A dissertation on

EXPRESSION OF E-CADHERIN AND VIMENTIN IN ORAL SQUAMOUS CELL CARCINOMA AND ITS CORRELATION WITH GRADING AND NODAL METASTASIS

Dissertation Submitted in

in partial fulfilment of the regulations required for the award of

M.D.DEGREE IN PATHOLOGY - BRANCH III

THE TAMILNADU

Dr.M.G.R. MEDICAL UNIVERSITY

CHENNAI

MAY 2019

DECLARATION

I hereby declare that the dissertation entitled "**EXPRESSION OF E-CADHERIN AND VIMENTIN IN ORAL SQUAMOUS CELL CARCINOMA AND ITS CORRELATION WITH GRADING AND NODAL METASTASIS**" is a bonafide research work done by me in the Department of Pathology, Coimbatore Medical College during the period from January 2017 to May 2018 under the guidance and supervision of **Dr.B.Sudha,M.D**., Senior Assistant Professor, Department of Pathology, Coimbatore Medical College.

This dissertation is submitted to the Tamilnadu Dr.M.G.R Medical University, Chennai towards the partial fulfilment of the requirement for the award of M.D. Degree (Branch III) in Pathology. I have not submitted this dissertation on any previous occasion to any university for the award of any Degree.

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Guide

Dr.B. Sudha, M.D.,
Senior Assistant Professor,
Department of Pathology,
Coimbatore Medical College,
Coimbatore.

Head of the Department

Dr.C.Lalitha,M.D.,
Professor,
Department of Pathology,
Coimbatore Medical College,
Coimbatore.

Dr.B. Asokan, M.S., M.ch.,
Dean,
Coimbatore Medical College,
Coimbatore.

Coimbatore Medical College

COIMBATORE, TAMILNADU, INDIA - 641 014

(Affiliated to The Tamilnadu Dr. MGR Medical University, Chennai)

ETHICS COMMITTEE

CERTIFICATE

Name of the Candidate:		**Dr. R. Vigneswari**
Course	:	**MD (Pathology) Post Graduate**
Period of Study	:	**1 year**
College	:	**Coimbatore Medical College & Hospital.**
Dissertation Topic	:	**Expression of e-cadherin and vimentin in oral squamous cell carcinoma and its correlation with grading and nodal metastasis.**

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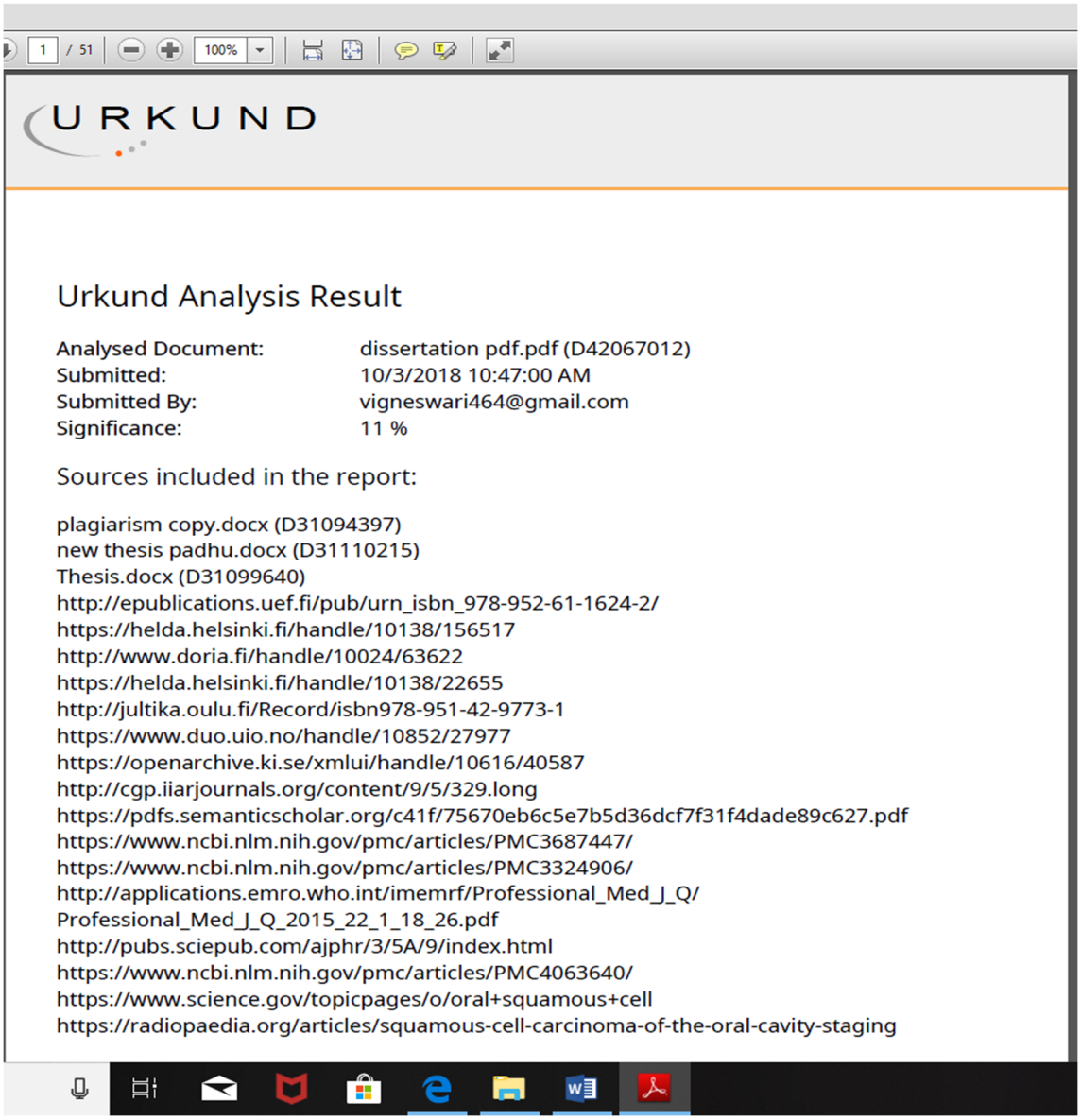

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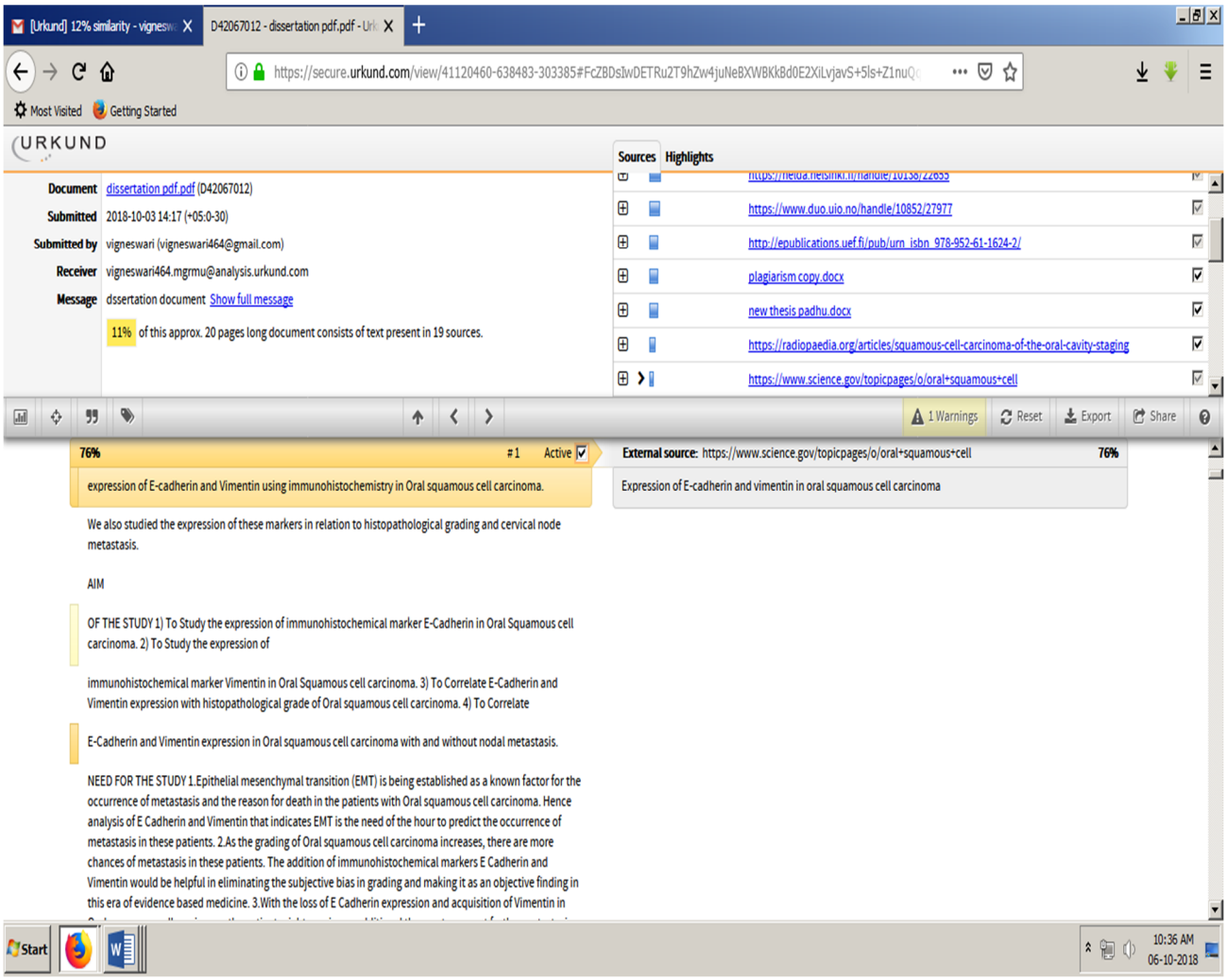
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Active
expression of E-cadherin and Vimentin using immunohistochemistry in Oral squamous cell carcinoma.
We also studied the expression of these markers in relation to histopathological grading and cervical node metastasis.
AIM
OF THE STUDY 1) To Study the expression of immunohistochemical marker E-Cadherin in Oral Squamous cell carcinoma. 2) To Study the expression of
immunohistochemical marker Vimentin in Oral Squamous cell carcinoma. 3) To Correlate E-Cadherin and Vimentin expression with histopathological grade of Oral squamous cell carcinoma. 4) To Correlate
E-Cadherin and Vimentin expression in Oral squamous cell carcinoma with and without nodal metastasis.
NEED FOR THE STUDY 1.Epithelial mesenchymal transition (EMT) is being established as a known factor for the occurrence of metastasis and the reason for death in the patients with Oral squamous cell carcinoma. Hence analysis of E Cadherin and Vimentin that indicates EMT is the need of the hour to predict the occurrence of metastasis in these patients. 2.As the grading of Oral squamous cell carcinoma increases, there are more chances of metastasis in these patients. The addition of immunohistochemical markers E Cadherin and Vimentin would be helpful in eliminating the subjective bias in grading and making it as an objective finding in this era of evidence based medicine. 3.With the loss of E Cadherin expression and acquisition of Vimentin in
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ACKNOWLEDGEMENT

I express my deep gratitude to **Dr.B.Asokan,M.S,M.ch.,** Dean, Coimbatore Medical College, for granting me permission to undertake this study.

I profusely thank and express my sincere gratitude to **Dr.C.Lalitha,M.D.,** Professor and Head, Department of Pathology, Coimbatore Medical College, for having rendered her valuable support and encouragement without which this project work would have not been feasible.

I express my heartful thanks to **Dr.B.Sudha,M.D.,** Senior Assistant Professor, Department of Pathology, Coimbatore Medical College, for having suggested this topic for dissertation, for her scholarly guidance, valuable advice throughout the course of this study.

I also wish to record my sincere thanks to all Associate Professors and Assistant Professors of the Department of Pathology, Coimbatore Medical College, for their constant support and encouragement throughout the work.

I thank all the technical staff in the Department of Pathology, Coimbatore Medical College, for their sincere and timely technical assistance.

Also, I am indebted to my parents, my husband **Dr.K.Gopalakrishnan,M.D.,** and colleagues for their moral support during this tenure. Last but not the least, I profusely thank all the patients from whom tissues were taken for the study.

Dr.R.Vigneswari

PLAGIARISM CERTIFICATE

This is to certify that this dissertation work titled "**EXPRESSION OF E-CADHERIN AND VIMENTIN IN ORAL SQUAMOUS CELL CARCINOMA AND ITS CORRELATION WITH GRADING AND NODAL METASTASIS"** of the candidate **R.Vigneswari** with registration number **201613257** for the award of **M.D degree** in the branch of **PATHOLOGY.** I personally verified the urkund.com website for the purpose of plagiarism check. I found that the uploaded thesis file contains from introduction to conclusion pages and result shows **11** percentage of plagiarism in the dissertation.

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INTRODUCTION

Oral cancer constitutes the sixth most prevalent cancer worldwide but ranks third in developing world and eighth in developed countries.[1] Oral cancer accounts for 5% of all cancers in men and 2% in women. Approximately 94% of all oral malignancies are Squamous cell carcinoma. In recent years, there is increase in incidence rates and mortality particularly in young adults as an outcome of this disease.[2]

Despite great improvement in adjunctive therapy and surgical treatment, 5-year survival rate of oral cancer has not improved over past few decades.[3-5]

Most important cause for poor survival rate is local or regional relapse and cervical lymph node metastasis. If reliable biomarker could be identified to predict metastasis and prognosis, it would aid in selection of definitive treatment strategy and could improve survival.

Only few studies have examined markers that would predict progression and metastasis [6,7] and molecular mechanisms mediating invasion. Therefore, there is a need to study molecular markers to

understand cancer progression and metastasis that would help in selecting more selective and suitable treatment for cancer.

Studies of epithelial malignancies have shown that loss of epithelial morphology and acquisition of mesenchymal characteristics termed as Epithelial mesenchymal transition (EMT) are typical for carcinoma cells and correlate with invasiveness and metastatic potential of tumour. Cells proceeding EMT exhibit down-regulation of epithelial markers such as E-cadherin and upregulation of mesenchymal markers such as Vimentin.[8-10]

E-cadherin is a calcium dependent transmembrane glycoprotein located in epithelial tissue responsible for cell-cell adhesion and plays role in establishing cell polarity and normal tissue architecture.[11] Loss of E-cadherin expression increases the mobility of epithelial cells and its ability for local invasion.

Vimentin is type III intermediate filament protein normally found in mesenchymal cells such as fibroblasts, endothelial cells, hematopoietic cells and glial cells. Its expression in oral epithelial cells has been associated with invasion and metastasis of tumour.[12,13]

So, in our study we analysed the expression of E-cadherin and Vimentin using immunohistochemistry in Oral squamous cell carcinoma. We also studied the expression of these markers in relation to histopathological grade and cervical node metastasis.

AIM OF THE STUDY

1) To Study the expression of immunohistochemical marker E-Cadherin in Oral Squamous cell carcinoma.

2) To Study the expression of immunohistochemical marker Vimentin in Oral Squamous cell carcinoma.

3) To Correlate E-Cadherin and Vimentin expression with histopathological grade of Oral squamous cell carcinoma.

4) To Correlate E-Cadherin and Vimentin expression in Oral squamous cell carcinoma with and without nodal metastasis.

NEED FOR THE STUDY

1. Epithelial mesenchymal transition (EMT) is being established as a known factor for the occurrence of metastasis and the reason for death in the patients with Oral squamous cell carcinoma. Hence analysis of E-Cadherin and Vimentin that indicates EMT is the need of the hour to predict the occurrence of metastasis in these patients.

2. As the grading of Oral squamous cell carcinoma increases, there are more chances of metastasis in these patients. The addition of immunohistochemical markers E-Cadherin and Vimentin would be helpful in eliminating the subjective bias in grading and making it as an objective finding in this era of evidence-based medicine.

3. With the loss of E-Cadherin expression and acquisition of Vimentin in Oral squamous cell carcinoma, the patient might require an additional therapy to prevent further metastasis that would increase their survival rate.

REVIEW OF LITERATURE

INCIDENCE OF ORAL SCC:

Head and neck squamous cell carcinoma accounts for 6,50,000 new cases annually. Oral squamous cell carcinoma accounts for more than 90% of total head and neck carcinoma.

In India, Oral squamous cell carcinoma accounts for 9.4 percent of all cancers with an incidence of 12.6 per lakh population.[14] 50 -70% of total cancer mortality in India occurs due to oral squamous cell carcinoma.In India, 5 people die every hour because of oral cancer.[15]

Despite advances in treatment including radiotherapy, chemotherapy and surgery the overall long-term survival is less than 50%. Decrease in long term survival is because most of the oral cancer is detected only at an advanced stage.

AGE DISTRIBUTION:

Incidence of the oral squamous cell carcinoma increases with age. Most of the oral cancer occurs between the age of 50 and 70 years.

Although primarily a disease of the middle and older age groups, younger population presenting with Oral Squamous Cell Carcinoma has increased in recent years.

The etiological factors associated with Oral cancer in this age group is attributed to alteration in behavioural and lifestyle patterns.

GENDER DISTRIBUTION:

Men are affected two to four times more than women because of heavier indulgence in both tobacco and alcohol habits in most countries.

In India, the highest rates of intraoral cancer may be found in women who chew tobacco heavily.

LOCATION:

The frequency of occurrence of squamous cell carcinomas within the oral cavity are as follows: lip, 45%; tongue, 16%; floor of mouth, 12%; buccal mucosa, 10%; lower gingiva, 12%; upper gingiva and hard palate, 5%. Of the lip tumours, over 90% involve the lower lip.

ETIOLOGY:

Tobacco consumption is the most important risk factor as it alone accounts for millions of cancer deaths annually. The relationship between

smoking and oral cancer has been established firmly by epidemiological studies.[16]

Another major risk factor for Oral cancer is the consumption of alcohol. Persons who smoke and drink have 10 times greater risk of developing oral cancer than who do not smoke, and drink.[17,18] Other risk factors are betel quid chewing and marijuana use.

Human Papilloma virus (HPV)[19] and Herpes simplex virus (HSV) have been established as causative agents in recent years. Candida also plays a role in initiation of Oral cancer. Poor oral hygiene and prolonged irritation from sharp teeth have also been viewed for their possible role in the development.[20]

MOLECULAR PATHOGENESIS OF ORAL SCC:

Oral squamous carcinoma evolves through a multistep process in which multiple genetic events occur that alter the normal functions of oncogenes and tumour suppressor genes.[21] This leads to increased production of growth factors or cell surface receptors, transcription factors and enhanced intracellular messenger signalling. In combination with loss of tumour suppressor activity, this leads to increased cell

proliferation, with loss of cell cohesion, and the ability to infiltrate local tissue and spread to distant sites.

Specific genetic alterations have been identified in oral squamous cell carcinoma and precancerous lesions of oral cavity.[22,23] Genetic alterations that occur during carcinogenesis includes point mutations, amplifications, rearrangements, and deletions. Point mutations (single base changes) in K-ras and p53 genes leads to overactivity or inactivity of gene products. Amplification and rearrangement affect excitatory pathway genes, whereas rearrangement can also inactivate inhibitory pathway genes.

Deletions of chromosome 3p, 5q, and 9p with 3q gain have been identified in well differentiated tumours, whereas in poorly differentiated tumours deletions of 4q, 8p, 11q, 13q, 18q, 21q and gains in 1p, 11q, 13, 19, and 22q were identified.[24] Loss of heterozygosity (LOH) at 9p21–p22 were reported in 72% of tumours.[25] Allelic imbalance within 3p24–26, 3p21, 3p13, and 9p21 at one or more loci was associated with reduced survival and 25 fold increase in mortality rate.[26] Allelic loss or imbalance in regions at 3p21.30–22, and 3p12.1–13, p53, DCC (deleted in colon carcinoma) were reported. In dysplastic areas adjacent to carcinoma, LOH at DCC were identified.[27] These studies demonstrated that allelic

imbalance which was present in initial lesion has increased as the carcinoma developed.

Inactivation of protein product p16INK4 by deletion and mutation in cyclin dependent kinase inhibitor 2/multiple tumour suppressor gene 1 (CDKN2/MTSI) mapped to chromosome 9p21-22 has been found in 33% of Oral Squamous Cell carcinoma. p16INK4 binds to and inhibits phosphorylation of pRb by cyclin dependent kinases CDK4 and CDK6.[28,29]

Aberrant expression of proto oncogene epidermal growth factor receptor (EGFR/c-erb 1), c-myc, int-2, members of the ras gene family,hst-1, PRAD-1, and bcl-1 also contribute towards cancer development.[30] Aberrant expression of transformation growth factor α (TGF-α) is reported to occur that stimulate cell proliferation by binding to EGFR in an autocrine and paracrine fashion. [23]

EGFR, the biological receptor of EGF and TGF- α is overexpressed in 30% of oral cancer. The interaction of the receptor with its ligands such as EGF and TGF-α initiates a cascade of events, that triggers intrinsic tyrosine kinase activity. Increased numbers of EGF receptors are associated with the aggressiveness of the tumour and poor

degree of differentiation.[31] Oral squamous carcinomas overexpressing EGFR exhibit a greater response to chemotherapy, when compared with EGFR negative tumours.[32]

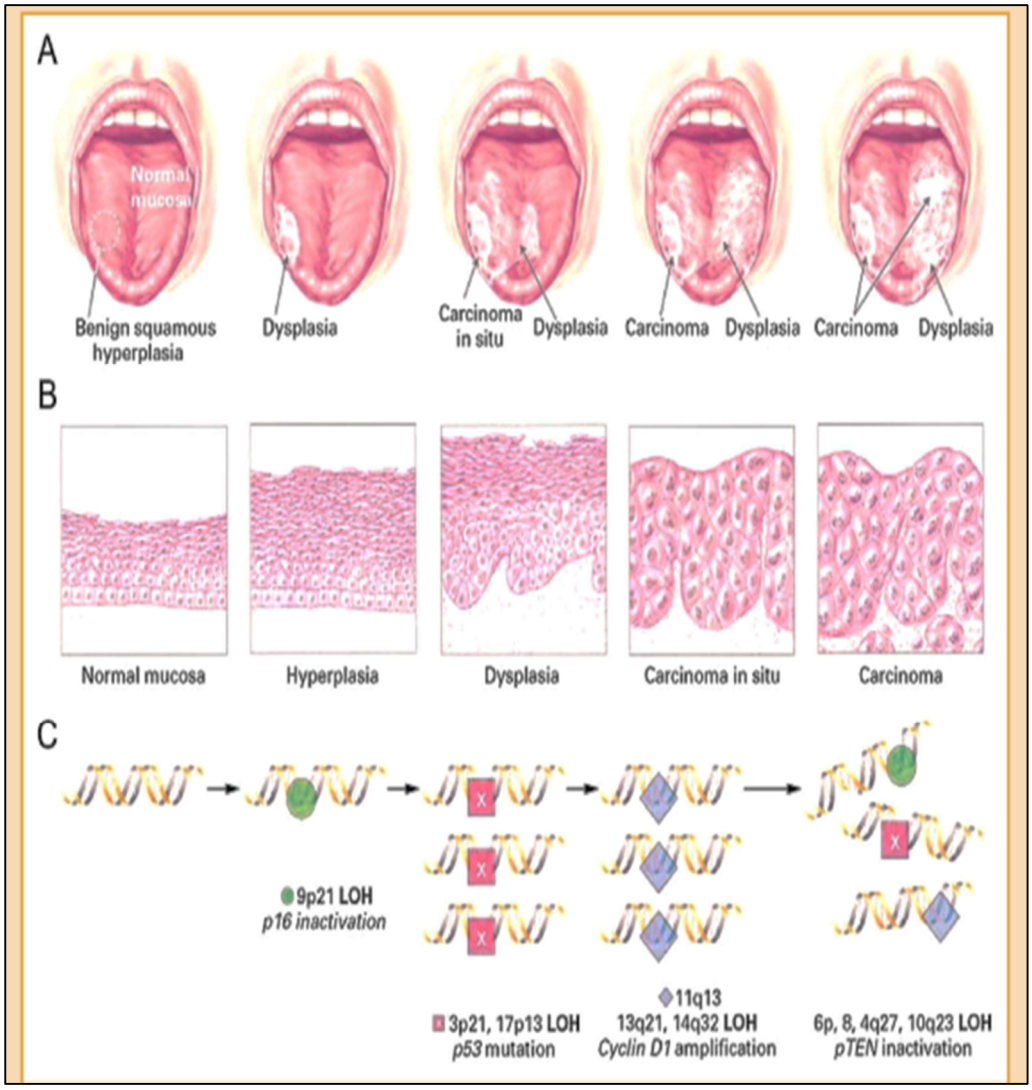


Figure 1: Progression model of multistep oral carcinogenesis.

Members of the ras family such as H-ras, K-ras, and N-ras which are involved in intracellular signalling pathways undergo mutation in oral

cancer. These genes encode protein p21 that transmits mitogenic signals by binding GTP. The mitogenic signal is terminated by conversion of GTP to GDP by hydrolysis .When the ras oncogene gets mutated, this conversion is prevented, leading to continuous stimulation.[33] C-Myc is overexpressed as a result of gene amplification in oral cancer.

C-Myc requires p53 for apoptosis and the retinoblastoma tumour suppressor gene Rb-1 nuclear protein pR6 interacts with c-myc gene, preventing its transcription, and thus inhibiting cell proliferation. However, when phosphorylation of pR6 occurs, c-Myc is increased and cell proliferation proceeds.[34]

The PRAD-1 gene on chromosome 11q13 encodes Cyclin D1, that together with Rb gene product controls the cell cycle in G1 to S transition. This gene is amplified in 30-50% of Oral cancers. Amplification of PRAD-1 is correlated with cytological grade, infiltrative growth and metastases.[35,36] Co amplification of the int-2 and hst-1 genes that encodes a protein similar to fibroblast growth factor is associated with tumour recurrence and progression.[37,38]

Alteration in p21 expression via p53 dependent and independent pathways is an early event in carcinogenesis of oral cancer[39]. Tobacco use

have been associated with mutation of p53 gene in Oral cancer. Activated STAT3 levels is elevated in oral cancer via up-regulation of the Jak, Src, EGFR, TGF-α, or interleukin-6 (IL-6) signalling pathways. In poorly differentiated oral cancers activated STAT3 is highly expressed and is correlated with metastasis. Loss of heterozygosity with mutation of the adenomatous polyposis coli (APC) tumour suppressor gene has been detected in 25% of Oral cancers.[40]

FIELD CANCERISATION:

The theory of field cancerisation states that the entire oral epithelium is at a risk of developing malignancy because of exposure to carcinogenic factors constantly and accumulation of genetic aberrations affecting oncogenes and tumour suppressor genes. This leads to the development of multiple primary tumour in upper aerodigestive tract. Because of such field cancerisation, individual with oral squamous cell carcinoma have 35% risk of developing new primary tumour in upper aerodigestive tract.

EPITHELIAL MESENCHYMAL TRANSITION IN ORAL SQUAMOUS CELL CARCINOMA:

The concept of epithelial and mesenchymal transition (EMT) was first proposed by Greenberg et al. Epithelial mesenchymal transition is a biological process in which an epithelial cell undergoes biologic metamorphosis to a mesenchymal phenotype to acquire increased resistance to apoptosis, improved migratory capacity, invasiveness and increased production of extracellular matrix components. The hallmark sign is the transformation of cohesive and polarized epithelial cells into mesenchymal-like cells that exhibit no polarization and high mobility. The completion of EMT is signalled by the degradation of underlying basement membrane and formation of a mesenchymal cell that migrate away from the epithelial layer in which it originated.

This process occurs during normal embryogenesis and organ development.[41] Epithelial mesenchymal transition is an essential component for inflammatory process and wound healing.[42] The process of EMT is associated with cancer metastasis and invasion, that correlates with poor prognostic markers such as poor tumour staging, cancer recurrence, and decrease in survival rate in several types of cancers.

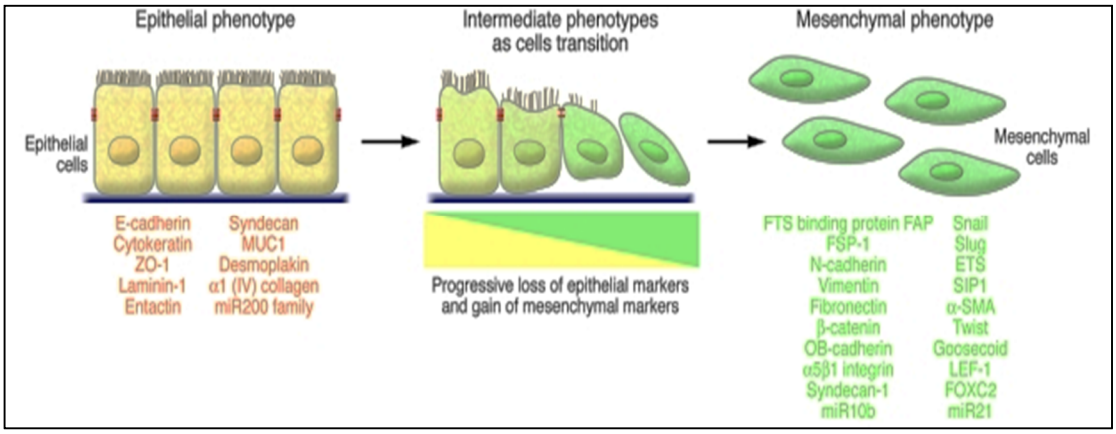


Figure 2: Epithelial mesenchymal transition

The phenomenon of Epithelial mesenchymal transition is divided into 3 types.[43]

Type 1 EMT: Type 1 EMT occurs during implantation, embryogenesis and development. The primitive epithelium, the epiblast gives rise to mesenchyme via an EMT. This mesenchyme is reinduced to form secondary epithelia by a MET (Mesenchymal epithelial transition). It is speculated that such secondary epithelia further differentiate to form other types of epithelial tissues and undergo subsequent EMT to generate cells of connective tissue such as astrocytes, adipocytes, chondrocytes, osteoblasts and muscle cells.

Type 2 EMT: This is associated with tissue regeneration and fibrosis. It is expressed over extended period and destroy an affected organ if primary insult is not removed.

Type 3 EMT: This type is associated with cancer progression and metastasis. The Secondary epithelia associated with many organs transform into cancer cells that later undergo the EMT that enable invasion and metastasis.

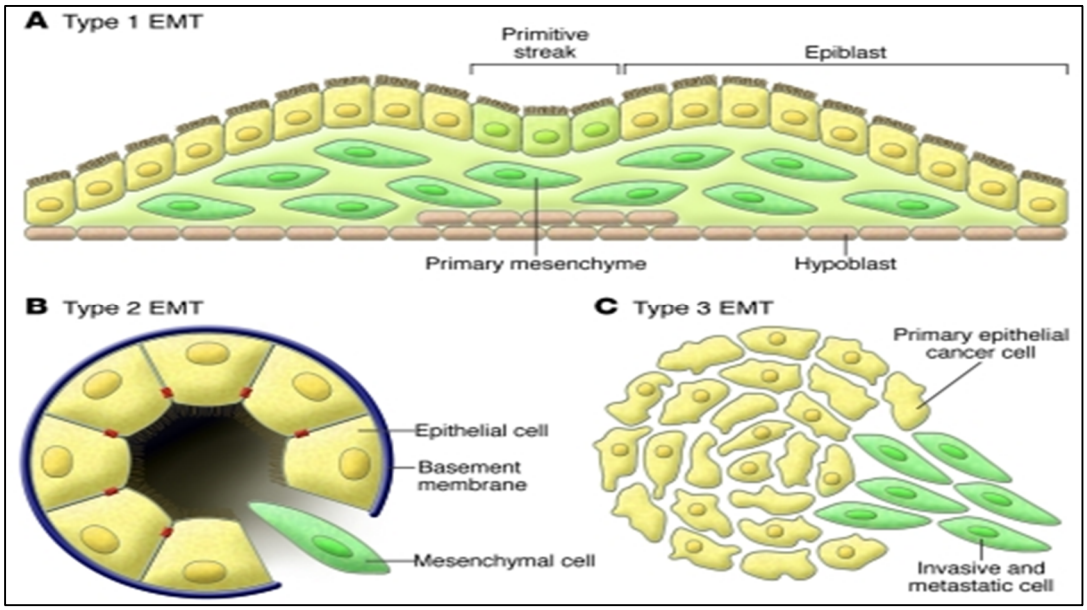


Figure 3: Different types of Epithelial mesenchymal transition

Many studies and experiments demonstrated that carcinomatous cells acquire mesenchymal phenotype and express mesenchymal markers such as FSP1, Vimentin, desmin and α-SMA.[44] These cells which are seen at invasive front of tumours eventually enter steps of the invasion-metastasis cascade such as intravasation, transport through circulation, extravasation, formation of micro metastases and colonization.[45,46,47]

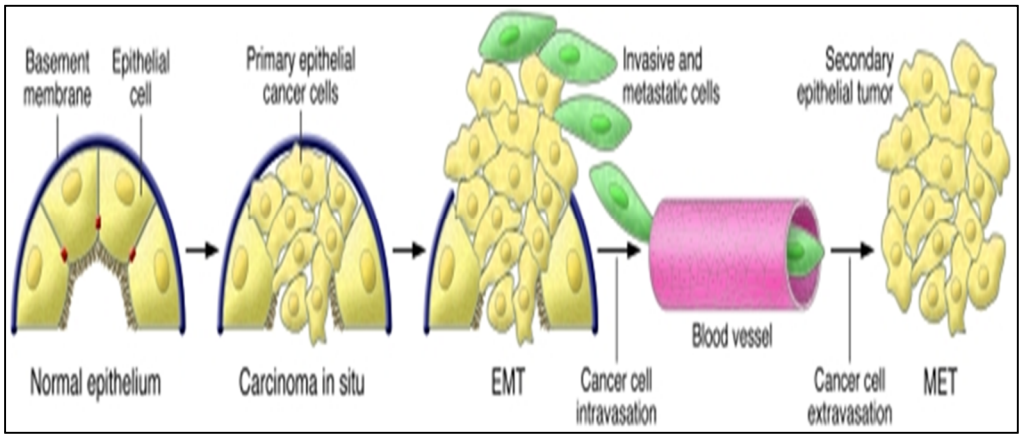


Figure 4: Contribution of EMT in cancer progression.

EMT-inducing signals from the tumour-associated stroma such as HGF, EGF, PDGF, and TGF-β are responsible for induction or functional activation in cancer cells of a EMT-inducing transcription factors such as Snail, Slug, Twist, zinc finger E-box binding homeobox 1(ZEB1), Goosecoid, and FOXC2.[48] The implementation of EMT program also depends on intracellular signalling networks involving Smads, RhoB, β-catenin ,ERK, MAPK, PI3K, Akt, lymphoid enhancer binding factor (LEF), Ras, and c-Fos and cell surface proteins such as β4 integrins, α5β1 integrin, and αVβ6 integrin.[49] Activation of EMT programs is also facilitated by disruption of cell-cell adherens junctions and cell-ECM adhesions mediated by integrins. TGF-β also induces EMT by two signalling pathways. One of these facilitates motility by involving Smad

proteins, which mediate TGF-β action to induce EMT via the ALK-5 receptor.

In oral squamous cell carcinoma epithelial markers such as E-cadherin, cytokeratin, Claudine, desmoplakin, beta keratin were down regulated and mesenchymal markers such as N-cadherin, vimentin, fibronectin, and snail-1/2 were up regulated by the process of EMT.

ROLE OF E- CADHERIN :

E-cadherin (Epithelial-cadherin), a calcium-dependent transmembrane glycoprotein of the type-1 cadherin family, is an important cell adhesion molecule and signal transduction factor in epithelial cells. It is encoded by the CDH1 gene that is located on chromosome 16q-22.1. It was first identified in chicken and was called originally as L-CAM.[50] E-cadherin is expressed at a very early two cell stage in embryogenesis where it plays an important role in adhesion of blastomeres.[51,52]

In adult epithelial tissues its function lies in formation of adherens junction, its maintenance and homeostasis.

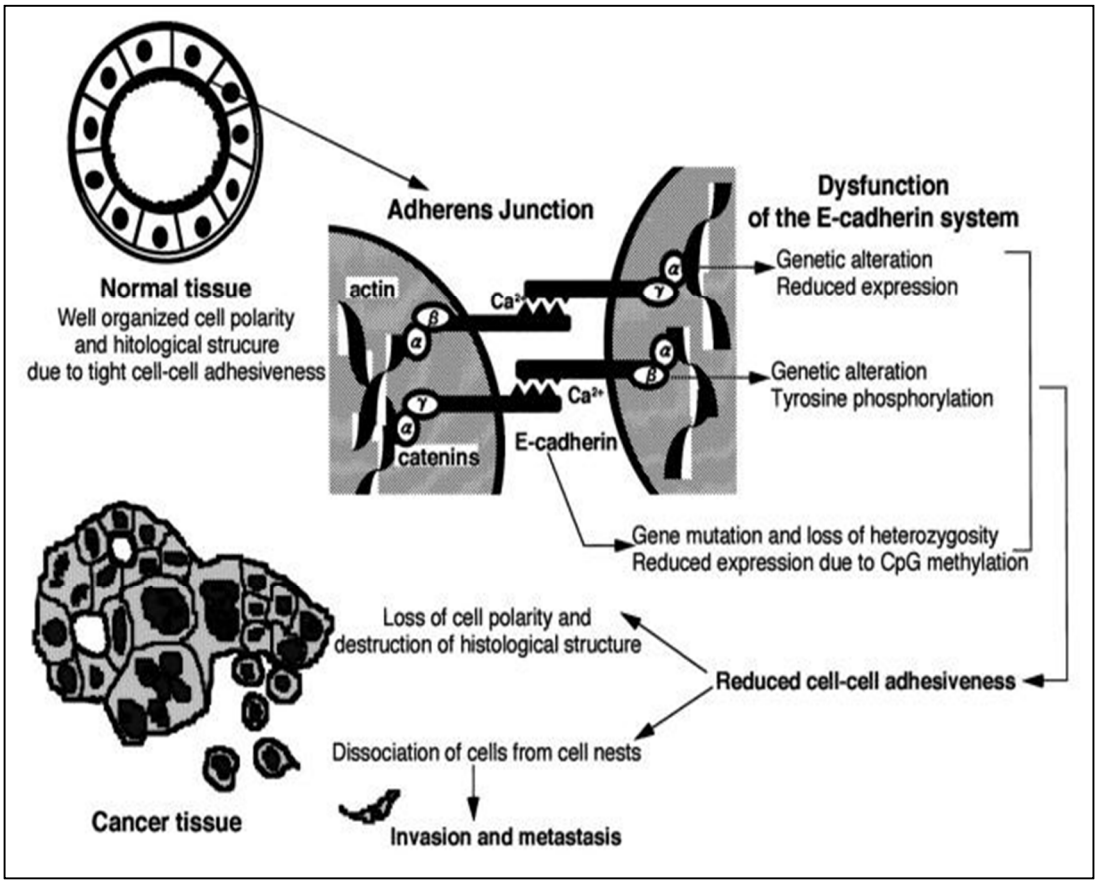


Figure 5:Role of E-cadherin in invasion and metastasis of tumour cells

In normal cells, E-cadherin exerts its tumour suppressing role mainly by sequestering β-catenin from its binding to LEF(Lymphoid enhancer factor)/ TCF (T cell factor). β-catenin serves the function of transcribing genes of the proliferative Wnt signalling pathway.

E-cadherin function can be altered at genetic and epigenetic levels. Examples of E-cadherin gene(CDH1 gene) alteration at genetic level are loss of heterozygosity (LOH) and inactivating mutations. Epigenetic

alterations that reduce cadherin expression includes promoter methylation, histone modifications and interplay of both that condenses chromatin conformation, limiting access to cis regulatory transcription factors, that control morphogenesis. MicroRNA (miRNA) dysregulation was discovered recently that play a part in epigenetic control of E-cadherin expression in cancer cells by targeting its multiple transcription regulators such as Snail 1, ZEB 1 and ZEB 2.

E cadherin as a tumour suppressor gene sets a threshold for Wnt/β-catenin signalling. When expression of E-cadherin is lost, potentiation of Wnt signalling pathway occurs leading to loss of cell-cell adhesion[53]. It is also postulated that blocking cadherin function causes decrease in receptor affinity of EGF-R and increased the surface mobility of EGF-R.

ROLE OF VIMENTIN

Vimentin, a 57 Kd protein is a type III intermediate filament protein normally found in mesenchymal cells such as fibroblasts, endothelial cells, hematopoietic cells, interstitial cells and glial cells . It is encoded by a single copy gene located on chromosome 10p13. It is sometimes expressed in migratory epithelial cells during embryogenesis and wound healing[12].The expression of vimentin in oral epithelial cells

induce several important features of EMT, including adoption of mesenchymal shape and increased motility by participating in signal transduction.

The epithelial cells expressing vimentin undergo flattening that facilitate these cells to withstand a variety of mechanical forces. This may be the critical factor for survival of metastatic cells when exposed to abnormal physical stress as they navigate from primary to secondary tumour sites.[54, 55]

Tumorigenic pathways of Vimentin are also encountered through its interaction with 14-3-3 and Akt phosphorylated Beclin 1 protein.[56] Upregulation of Vimentin also occurs through hypoxia-inducible factor-1 that contributes to the invasiveness of cancer cells. Vimentin regulate Axl expression, which is a receptor tyrosine kinase that increases cell motility. This is found by the studies which shows that silencing of Vimentin results in downregulation of Axl on mRNA and protein levels and subsequent inhibition of cell motility.

HISTOPATHOLOGY OF ORAL SQUAMOUS CELL CARCINOMA:

OSCC is a malignant neoplasm derived from the stratified squamous epithelium of the oral mucosa[57]. SCC can be ulcerative, papillary, flat or exophytic in growth. It ranges from minute mucosal thickenings to large masses that fills the luminal spaces.

During the process of carcinogenesis, the epithelium undergoes reactive epithelial changes such as hyperkeratosis, hyperplasia and acanthosis or preneoplastic changes (including mild, moderate and severe dysplasia) prior to the development of an invasive carcinoma . The precancerous lesions that progress to SCC are erythroplakia, leucoplakia, actinic cheilitis, lichen planus, sideropenic dysphagia, submucous fibrosis, dyskeratosis congenita, discoid lupus erythematosus.

The characteristic dysplastic features are;

1. Drop shaped rete pegs
2. Loss of cellular adhesion and cohesion
3. Disturbed polarity of basal cells
4. Basal cell hyperplasia

5. Irregular epithelial stratification or disturbed maturational sequence

6. Abnormal intraepithelial keratinization

7. Cellular pleomorphism/anisocytosis

8. Increase in nuclear cytoplasmic ratio

9. Nuclear hyperchromatism

10. Prominent nucleoli

11. Increased mitosis and abnormal mitosis .[58,59,60]

Conventional squamous cell carcinoma is composed of variable degrees of squamous differentiation and basement membrane violation by nests of tumour cells. In addition to the above-mentioned dysplastic features, malignant epithelial cells show keratin pearls and intercellular bridges. Inflammatory infiltrate (usually of lymphocytes and plasma cells) is seen at the tumour stroma junction, along with dense, desmoplastic fibrous stroma.

Squamous cell carcinoma is graded histologically for prediction of clinical and biological behaviour of tumour.

GRADING SYSTEMS

In 1920, Border developed a quantitative grading of 4 categories based on degree of differentiation. Jakobson et al developed a multifactorial grading system which was later modified by Anneroth and Hansen for application to squamous cell carcinoma of tongue and floor of mouth.

Border's grading system

Grade I: Well differentiated = <25% of undifferentiated cells

Grade II: Moderately differentiated =25-50% of undifferentiated cells

Grade III: Poorly differentiated =50-75% of undifferentiated cells

Grade IV: Anaplastic or pleomorphic =>75% of undifferentiated cells

Anneroth grading included six parameters such as degree of keratinization, nuclear pleomorphism, number of mitosis, pattern of invasion, stage of invasion, lympho-plasmocytic infiltration.

Akhter et al., observed that Anneroth's classification is a better predictor of lymph node metastasis. In 1989, Bryne proposed that

invasive tumour front grading system gave prognosis better than other areas of tumour.

According to **WHO** grading system, 3 categories are recommended :

- Well differentiated,
- Moderately differentiated and
- Poorly differentiated.

It depends on assessment of keratinisation, pleomorphism and mitotic activity.

VARIANTS OF SQUAMOUS CELL CARCINOMA:

VERRUCOUS SQUAMOUS CELL CARCINOMA (ACKERMAN'S TUMOR):

Verrucous carcinoma comprises about 3% of all SCC. It is more common among older women. Active pathogenetic role has been ascribed to human papilloma virus. Grossly it appears as a broad-based, warty, exophytic or fungating, bulky, firm to hard, tan or white mass measuring up to 10cm in greatest dimension. They may show surface ulceration.

Microscopically, verrucous squamous cell carcinoma is a highly differentiated type, composed of exophytic, warty tumour with multiple

filiform projections lined by well-differentiated squamous epithelium. The advancing margins of tumour are broad or bulbous rete pegs with pushing rather than infiltrative appearance and dense inflammation in adjacent tissues. There is an orderly maturation of cells towards the surface, with abundant surface keratosis called ‘church-spire Keratosis. Parakeratotic crypting is a common feature. Mitotic figures are rare and focal atypia/dysplasia is limited to basal zone if present.

EXOPHYTIC AND PAPILLARY SQUAMOUS CELL CARCINOMA:

Exophytic and Papillary Squamous cell carcinoma are de novo malignancies without pre- or co-existing benign lesion(squamous papilloma).[61] The average size of exophytic and papillary tumours is about 1.5 and 1cm in greatest dimension. Grossly they are polypoid, exophytic, bulky, papillary or fungiform tumours, soft to firm, arising from a broad base or from a narrow pedicle/stalk[62,63,64]. The neoplastic squamous epithelial proliferation must demonstrate a dominant (> 70%) exophytic or papillary architectural growth pattern with unequivocal cytomorphologic evidence of malignancy.

Two specific histologic growth patterns are identified that separates from conventional SCC. The exophytic pattern is composed of broad based, bulbous to exophytic growth of squamous epithelium. The projections are ‘cauliflower-like’ and are rounded. Central fibrovascular cores are seen on tangential sectioning, but the superficial aspect of growth is lobular and not papillary.

The papillary pattern is composed of multiple, thin, delicate filiform, finger-like papillary projections. The papillae consist of a delicate fibrovascular core surrounded by the neoplastic epithelium. On tangential sectioning it shows several central fibrovascular cores, appearing like a bunch of celery cut across the stalk. Both exophytic and papillary shows features of SCC such as focal surface keratinization, dyskeratosis, architectural distortion with loss of cellular polarity, nuclear enlargement, increased nuclear to cytoplasmic ratio, prominent nucleoli and numerous mitotic figures. The invasion is usually superficial. No perineural, vascular or osseous invasion are seen. So-called ‘koilocytic atypia’ defined by hyperchromatic, crenated nuclei surrounded by a clear halo of cytoplasm and an accentuated cell border is frequently noted due to pathogenetic role played by Human papilloma virus.

SPINDLE CELL (SARCOMATOID CARCINOMA):

It is also known as Carcinosarcoma, Pseudo sarcoma, Squamous cell carcinoma with pseudo sarcoma, Lane tumour over the years. Spindle cell carcinoma is recognized as biphasic tumour with carcinoma that has surface epithelial changes (dysplasia to invasive carcinoma) and an underlying spindle-shaped neoplastic proliferation.

It comprises about 3% of Squamous cell carcinoma. There is an increase in male to female ratio. Radiation exposure may be an etiologic agent. All cases present as polypoid masses with a mean size of 2 cm. They are frequently ulcerated with a firm and fibrous cut surface. Histologically sarcomatous part predominates but dysplasia, carcinoma in-situ, or infiltrating SCC can be identified. Areas of squamous differentiation are identified at the base of the lesion or within invagination at the surface where the epithelium is not ulcerated or denuded.[65,66] The carcinomatous and sarcomatous components will abut against one another, with barely any areas of perceptible blending. The Sarcomatous component shows diverse array of appearance such as storiform, cartwheel, or whorled intersecting and interlacing bundles or fascicles and chevron or herringbone pattern. The squamous component

includes plump fusiform, rounded and epithelioid cells. Opacified, dense, eosinophilic cytoplasm give a hint of squamous differentiation.

BASALOID SQUAMOUS CELL CARCINOMA:

Basaloid squamous cell carcinoma is a high-grade variant of squamous cell carcinoma. It affects men in seventh decade of life with frequent cervical lymph node metastasis. Grossly they present as exophytic to nodular masses measuring up to 6 cm in greatest dimension. They are firm to hard and associated with central necrosis.

Histologically, the infiltrating tumour shows a variety of growth patterns, such as solid, lobular, cribriform, cords, trabeculae, nests and glands or cysts. Surface ulceration is frequently noted. Basaloid component is defined by features that includes

1) Solid growth of cells in a lobular configuration with peripheral palisading, closely associated with or involving surface mucosa.

2) Individual cells are small, crowded with scant cytoplasm having dark hyperchromatic nuclei without nucleoli.

3) Small cystic spaces containing material resembling mucin that stains with periodic acid-Schiff or alcian blue.[67,68,69]

The lobules often contain central necrosis with visible necrotic material. Sometimes the necrotic material completely "drops out," giving a pseudo glandular appearance . These basaloid regions are in direct continuity with areas of squamous differentiation, including abrupt keratinization in the form of squamous pearls, individual cell keratinization, etc.[70]

ADENOSQUAMOUS CARCINOMA:

Adenosquamous carcinoma is a high-grade variant of squamous cell carcinoma with admixture of both squamous cell carcinoma and adenocarcinoma. They often present as an indurated submucosal nodule ranging in size from less than 1 cm to 5 cm in maximum dimension. Most patients present with lymph node metastasis. Any two of the following squamous features are mandatory:

1) Intercellular bridging
2) Keratin pearl formation
3) Parakeratotic differentiation
4) Individual cell keratinization
5) Cellular arrangements showing pavement or mosaic pattern.

The adenocarcinoma component can be tubular, alveolar and/or glandular. The cells in adenocarcinoma can also be basaloid. The glandular epithelium requires the demonstration of intracytoplasmic sialomucin by high iron diamine alcian blue or PAS stain retention after diastase digestion or Mayer's mucicarmine.

The tumour cells were of the three basic types:

1) Basaloid cells
2) Squamous cells
3) Undifferentiated cells.

The malignant cells usually demonstrate frequent mitoses, necrosis and infiltration into the surrounding tissue with perineural invasion. There is sparse inflammatory cell infiltrate at the tumour-stromal interface.[71-74]

SPREAD AND METASTASIS:

The pattern of spread of oral squamous cell carcinoma is dictated by the anatomic features of the primary site.[75] Carcinoma of the lip invades adjacent skin, orbicular muscle, buccal mucosa, the adjacent mandible and mental nerve. Tumours of the floor of the mouth penetrate beneath the mucosa into the sublingual gland, into the midline muscles, and extend toward the gingiva and mandible.[76] Tumours of the tongue,

that usually arise on the lateral surfaces and under surfaces, remain localized for long periods but eventually invade the floor of the mouth and root of the tongue, resulting in fixation of the organ. Tumours of the buccal mucosa invade the underlying muscles and may eventually penetrate the skin. Tumours of gingiva extend quickly into the periosteum, the adjacent buccal mucosa, and the floor of mouth. Tumours of the hard palate may spread into the underlying bone, but extension into the maxillary antrum is very rare. Tumours of the retromolar trigone spread to adjacent buccal mucosa, anterior tonsillar pillar, maxilla, pterygomandibular space, medial pterygoid muscle, and buccinator muscle.

SCC of the oral cavity predominantly metastasizes to the lymph nodes of the neck. The site of the involved nodes depends on the location of the primary tumor.[77] The prognosis being diminished by half if lymph node metastases are present at presentation. Extracapsular spread and the presence of a desmoplastic stromal response in tumour-positive lymph nodes has been shown to worsen prognosis.[78] The involvement of neck node also correlates with an increased risk of development of distant metastases.[79,80,81]

PROGNOSIS:

Following are the prognostic determinants of oral cavity:

1. Location:

The overall 5-year survival rates are about 90% for carcinomas of the lower lip; 60% for tumours of the anterior tongue; 40% for tumours of the posterior tongue, floor of mouth, tonsil, gingiva, and hard palate; and 20–30% for tumours of the soft palate. However, these figures are heavily influenced by tumour stage.[82-86]

2. Stage:

5-year survival rate of patients with stage I, 91.0%; stage II, 77.2%; stage III, 61.2%; stage IVA, 32.4%; stage IVB, 25.3%; stage IVC, 3.6%.[87]

3. Grade:

It is an independent prognostic factor. Grading of the deep invasive margins of the tumour provides better prognostic information than grading of the entire tumor.[88]

4. Depth of invasion:

This is an important factor, in some locations.[89] This feature is incorporated into the staging systems.

5. Tumour size:

Size of the tumour does not correlate closely with clinical outcome except in small tumors.[90]

6. Desmoplastic reaction:

Presence of a florid desmoplastic reaction has been found to be a marker of aggressive behaviour with higher likelihood of metastases.[91]

7. Tissue eosinophilia:

Intense infiltration of the carcinoma by eosinophils is said to be a favourable prognostic factor.[92]

8. Lymph node involvement:

Presence of lymph node metastases is an important prognostic criterion and it is a key feature of the staging system.[93] Extracapsular spread (i.e., spread of the metastases beyond the lymph node capsule) is an indicator of a further decrease in survival rates.[94]

9. DNA ploidy:

The nondiploid tumours tend to be clinically more advanced than the diploid ones.[95] DNA ploidy correlates with the microscopic grade of the tumour and with prognosis.

10. HPV-16:

HPV-16 positivity in squamous cell carcinomas of the oral cavity is a powerful indicator of improved survival.[96]

11. H antigen:

It has been observed that loss of expression of blood group antigen is associated with a greater tendency for invasiveness and distant spread.[97]

12. p21 gene:

Overexpression of p21 gene (the product of which is the downstream regulatory protein of TP53) was found to be an unfavourable prognostic factor in lingual squamous cell carcinoma.[98]

13. 3q26.3 locus:

Amplification of this genetic locus found to be associated with tumour progression and poor prognosis.[99]

14. TROP2:

Overexpression of this human trophoblast cell-surface antigen has found to be associated with decreased overall survival.[100]

15. p16:

Overexpression of p16 was found to be a favourable prognostic factor as it is a surrogate marker for high-risk HPV, a known favourable prognostic factor.[101]

WHO CLASSIFICATION OF ORAL CAVITY AND OROPHARYNX

MALIGNANT EPITHELIAL TUMOURS:

Squamous cell carcinoma

Verrucous carcinoma

Basaloid squamous cell carcinoma

Papillary squamous cell carcinoma

Spindle cell carcinoma

Acantholytic squamous cell carcinoma

Adenosquamous carcinoma

Carcinoma cuniculatum

Lymphoepithelial carcinoma

EPITHELIAL PRECURSOR LESIONS

BENIGN EPITHELIAL TUMOURS

Papillomas

- Squamous cell papilloma and verruca vulgaris
- Condyloma acuminatum
- Focal epithelial hyperplasia

Granular cell tumour

Keratoacanthoma

SALIVARY GLAND TUMOURS

Salivary gland carcinomas

- Acinic cell carcinoma
- Mucoepidermoid carcinoma
- Adenoid cystic carcinoma
- Polymorphous low-grade adenocarcinoma
- Basal cell adenocarcinoma
- Epithelial-myoepithelial carcinoma
- Clear cell carcinoma not otherwise specified
- Cystadenocarcinoma

Mucinous adenocarcinoma

Oncocytic carcinoma

Salivary duct carcinoma

Myoepithelial carcinoma

Carcinoma ex pleomorphic adenoma

Salivary gland adenomas

Pleomorphic adenoma

Myoepithelioma

Basal cell adenoma

Canalicular adenoma

Duct papilloma

Cystadenoma

SOFT TISSUE TUMOURS

Kaposi sarcoma

Lymphangioma

Ectomesenchymal chondromyxoid tumour

Focal oral mucinosis

Congenital granular cell epulis

HAEMATOLYMPHOID TUMOURS

Diffuse large B-cell lymphoma (DLBCL)

Mantle cell lymphoma

Follicular lymphoma

Extra nodal marginal zone B-cell lymphoma of MALT type

Burkitt lymphoma

T-cell lymphoma (including anaplastic large cell lymphoma)

Extramedullary plasmacytoma

Langerhans cell histiocytosis

Extramedullary myeloid sarcoma

Follicular dendritic cell sarcoma / tumour

MUCOSAL MALIGNANT MELANOMA

SECONDARY TUMOURS

TNM CLASSIFICATION OF CARCINOMAS OF THE ORAL CAVITY AND OROPHARYNX

TNM CLASSIFICATION OF CARCINOMAS OF THE LIP AND ORAL CAVITY

T Primary tumour

TX Primary tumour cannot be assessed

T0 No evidence of primary tumour

Tis Carcinoma in situ

T1 Tumour 2 cm or less in greatest dimension

T2 Tumour more than 2 cm but not more than 4 cm in greatest dimension

T3 Tumour more than 4 cm in greatest dimension

T4a (lip) Tumour invades through cortical bone, inferior alveolar nerve, floor of mouth, or skin (chin or nose)

T4a (oral cavity) Tumour invades through cortical bone, into deep/extrinsic muscle of tongue (genioglossus, hyoglossus, palatoglossus, and styloglossus), maxillary sinus, or skin of face

T4b (lip and oral cavity) Tumour invades masticator space, pterygoid plates, or skull base; or encases internal carotid artery

Note: Superficial erosion alone of bone/tooth socket by gingival primary is not sufficient to classify a tumour as T4.

N – Regional lymph nodes##

NX Regional lymph nodes cannot be assessed

N0 No regional lymph node metastasis

N1 Metastasis in a single ipsilateral lymph node, 3 cm or less in greatest dimension

N2 Metastasis as specified in N2a, 2b, 2c below

N2a Metastasis in a single ipsilateral lymph node, more than 3 cm but not more than 6 cm in greatest dimension

N2b Metastasis in multiple ipsilateral lymph nodes, none more than 6 cm in greatest dimension

N2c Metastasis in bilateral or contralateral lymph nodes, none more than 6 cm in greatest dimension

N3 Metastasis in a lymph node more than 6 cm in greatest dimension

Note: Midline nodes are considered ipsilateral nodes.

M Distant metastasis

MX Distant metastasis cannot be assessed

M0 No distant metastasis

M1 Distant metastasis

Stage grouping

Stage 0	Tis,N0,M0
Stage I	T1, N0, M0
Stage II	T2, N0, M0
Stage III	T1/ T2,N1, M0
	T3,N0/ N1, M0
Stage IVA	T1/ T2/ T3, N2, M0
	T4a,N0/N1/N2,M0
Stage IVB	Any T, N3, M0
	T4b ,Any N, M0
Stage IVC	Any T, Any N, M1

The regional lymph nodes are the cervical nodes.

TNM CLASSIFICATION OF CARCINOMAS OF THE OROPHARYNX

T Primary tumour

TX Primary tumour cannot be assessed

T0 No evidence of primary tumour

Tis Carcinoma in situ

T1 Tumour 2 cm or less in greatest dimension

T2 Tumour more than 2 cm but not more than 4 cm in greatest dimension

T3 Tumour more than 4 cm in greatest dimension

T4a Tumour invades any of the following: larynx, deep/extrinsic muscle of tongue (genioglossus, hyoglossus, palatoglossus, and styloglossus), medial pterygoid, hard palate, and mandible

T4b Tumour invades any of the following: lateral pterygoid muscle, pterygoid plates, lateral nasopharynx, skull base; or encases the carotid artery

N – Regional lymph nodes##

NX Regional lymph nodes cannot be assessed

N0 No regional lymph node metastasis

N1 Metastasis in a single ipsilateral lymph node, 3 cm or less in greatest dimension

N2 Metastasis as specified in N2a, 2b, 2c below

N2a Metastasis in a single ipsilateral lymph node, more than 3 cm but not more than 6 cm in greatest dimension

N2b Metastasis in multiple ipsilateral lymph nodes, none more than 6 cm in greatest dimension

N2c Metastasis in bilateral or contralateral lymph nodes, none more than 6cm in greatest dimension

N3 Metastasis in a lymph node more than 6 cm in greatest dimension

Note: Midline nodes are considered ipsilateral nodes.

M Distant metastasis

MX Distant metastasis cannot be assessed

M0 No distant metastasis

M1 Distant metastasis

Stage grouping

Stage 0	Tis, N0, M0
Stage I	T1, N0, M0
Stage II	T2, N0, M0
Stage III	T1/ T2, N1, M0
	T3, N0/ N1, M0
Stage IVA	T1/ T2/ T3, N2, M0
	T4a, N0/ N1/ N2, M0
Stage IVB	T4b, Any N, M0
	Any T N3 M0
Stage IVC	Any T, Any N, M1

The regional lymph nodes are the cervical nodes.

MATERIALS AND METHODS

STUDY CHARACTERISTICS:

Forty patients with Oral squamous cell carcinoma who attended the outpatient and inpatient department of Coimbatore Medical College Hospital between January 2017 to May 2018 were included in the study. The Immunoexpression of molecular markers (E-cadherin and Vimentin) were studied in their tissue sections and correlated with degree of differentiation of tumour and its association with cervical lymph node metastasis was analysed.

PATIENT CHARACTERISTICS:

Patients diagnosed with Oral squamous cell carcinoma in tongue, floor of mouth, buccal mucosa, lip, alveolar margin, tonsil, retromolar trigone and palate were included in the study. Patients who presented with lymph node involvement were also included in the study. Patients suffering from other primary malignancies, systemic illness and antenatal patients were excluded from the study.

SAMPLES:

Of the 40 patients with Oral squamous cell carcinoma , 20 patients presented with cervical lymph node metastasis. Split up of the specimens showed their origin to be 11 from tongue, 7 each from buccal mucosa and tonsillar fossa, 5 from lip, 4 from retromolar trigone, 3 from soft palate, 2 from alveolar margin and 1 from floor of mouth.

For histopathological and immunohistochemical studies the tumour samples were fixed in 10% buffered formalin, processed and then embedded in paraffin. The diagnosis was confirmed by routine histopathological examination using haematoxylin and eosin stain. For immunohistochemical studies, 4 micrometer thick tissue sections were taken in specially coated slides using chrome-alum and gelatin. The tissue sections included not only tumour lesions but also the adjacent non-tumorous oral epithelium in few cases that served as internal controls for immunochemistry.

In Patients with enlarged cervical nodes, fine needle aspiration was done, and the cytology smear was examined to confirm the presence of metastatic deposit.

IMMUNOHISTOCHEMISTRY:

PRINCIPLE:

The demonstration of antigen in cells and tissues by immunostaining is a twostep process. First is the binding of antibody to antigen of interest. Second is the detection of bound antibody by one of the varieties of enzyme chromogenic system. In our study we have used " The Super Sensitive polymer-HRP Detection System" that is based on a non-biotin polymeric technology that makes use of two major components: Super Enhancer and a Poly-HRP reagent. The problems associated with endogenous biotin are eliminated as the system is not based on Biotin-Avidin system. In this technique a large number of peroxidase enzyme molecules are bound to secondary antibody through the dextran backbone. This was done to increase the sensitivity.

SIMPLE PROTOCOL:

1. Application of primary antibody
2. Application of enzyme labelled polymer.
3. Application of the substrate chromogen.

REAGENTS USED:

1. Peroxide block: 3% hydrogen peroxide in water.
2. Power block reagent: A highly effective Universal protein blocking reagent. Contains casein and propriety additives in PBS with 15mM sodium azide.
3. Chromogen: DAB-3,3'-diaminobenzidine
4. Liquid DAB substrate: Tris buffer containing peroxide and stabilizers.
5. Super Enhancer Reagent.
6. Poly-HRP Reagent
7. Counterstain: Ehrlich's Haematoxylin
8. Buffer solutions

TRIS BUFFER (pH 7.6)

TRIS Buffer salt : 0.605 gm

Sodium chloride : 8 gm

Distilled water : 1000 ml

1 N Hydrochloric acid : 3 ml

CITRATE BUFFER (Ph 6.0)

Trisodium citrate : 2.94 gm

Distilled water : 1000 ml

1 N Hydrochloric acid : 5 ml

TRIS EDTA: (Ph 9.0)

TRIS Buffer salt : 6.05 gm

Disodium EDTA : 0.744 gm

Distilled water : 1000 ml

IMMUNOHISTOCHEMISTRY PROCEDURE:

1) Sections were cut at 4 microns, taken in a coated slide & incubated at 58C Overnight.
2) Deparaffinized in Xylene for 30 minutes.
3) Immersed in absolute alcohol for 2 minutes with 2 changes.
4) Washed in tap water for 10 minutes.
5) Rinsed in distilled water for 5 minutes.
6) Antigen retrieval was done by placing the slides with appropriate buffer solutions (Tris buffer for E-cadherin and citrate for vimentin) in microwave: Medium energy 10 minutes and high energy 10 minutes.

7) Cooled to room temperature for 20 minutes.

8) Rinsed in distilled water for 5 minutes.

9) Washed in TBS wash buffer for 5 minutes with 2 changes.

10) The Sections were covered with peroxide block for 10 minutes.

11) Washed in TBS wash buffer for 5 minutes with 2 changes.

12) The Sections were covered with power block for 10 minutes.

13) The Sections were drained and covered with primary antibody without washing (E-Cadherin and Vimentin for 1 hour) and kept in a moisture chamber.

14) Washed in TBS wash buffer 5 minutes with 2 changes.

15) The Sections were covered with Super enhancer for 30 minutes.

16) Washed in TBS wash buffer for 5 minutes with 2 changes.

17) The Sections were covered with S.S label + poly HRP for 30 minutes.

18) Washed in TBS wash buffer for 5 minutes with 2 changes.

19) The Sections were covered with chromogen (DAB + Substrate buffer) for 5 to 8 minutes.

20) Washed in TBS buffer for 5 minutes with 2 changes.

21) Washed in tap water for 5 minutes.

22) Counter stained with haematoxylin for 30 seconds.

23) Washed in tap water for 5 minutes.

24) Air dried, Cleaned in Xylene and mounted with DPX mountant.

RESULT: the development of brown colour was interpreted as positive scoring was done using the method mentioned below.

EVALUATION OF IMMUOHISTOCHEMICAL STAINING:

The most representative tumour areas were selected for scoring immunostaining pattern. The scoring was done using light microscopy. The following criteria were used to study the distribution and intensity of positive tumour cell staining. Cell membrane staining was considered as strong positive reaction and Cytoplasmic training as weak positive reaction for E-cadherin. Cytoplasmic staining was considered as positive reaction for Vimentin.

Immunoreactivity for E-cadherin and Vimentin were evaluated based on staining intensity and distribution using immunoreactive score.

Immunoreactive Score = Intensity Score X Proportion Score

INTENSITY SCORE :

Intensity score was defined as

SCORE	EXPRESSION
0	Negative
1	Weak
2	Moderate
3	Strong

PROPORTION SCORE Proportion score was defined as

SCORE	EXPRESSION
0	Negative
1	< 10% of the tumour cells
2	10-20% of the tumour cells
3	20-50% of the tumour cells
4	>50% of the tumour cells

Immunoreactivity score = Proportion score X Intensity Score

The total score ranged from 0 to 12.

The Immunoreactivity score was divided into three groups on the basis of final score.

TOTAL SCORE	IMMUNOREACTIVITY
0	Negative
1 – 4	Low
> 4(5-12)	High

OBSERVATION AND RESULTS

RESULTS

Table I: Mean age of the study

	N	MINIMUM	MAXIMUM	MEAN±SD
AGE	40	37	70	55.50±8.8

Table II: Demographics of the population based on Age

AGE OF PATIENT	NUMBER OF CASES	PERCENTAGE
31 – 40 Years	4	10%
41 – 50 Years	8	20%
51 – 60 Years	17	42.5%
61 – 70 Years	11	27.5%

Table II demonstrates the age of patients suffering from oral squamous cell carcinoma included in our study. The youngest age of presentation in our study was 37 years and the eldest was 70 years. Predominant population (42.5%) fall under the age group of 51- 60 years.

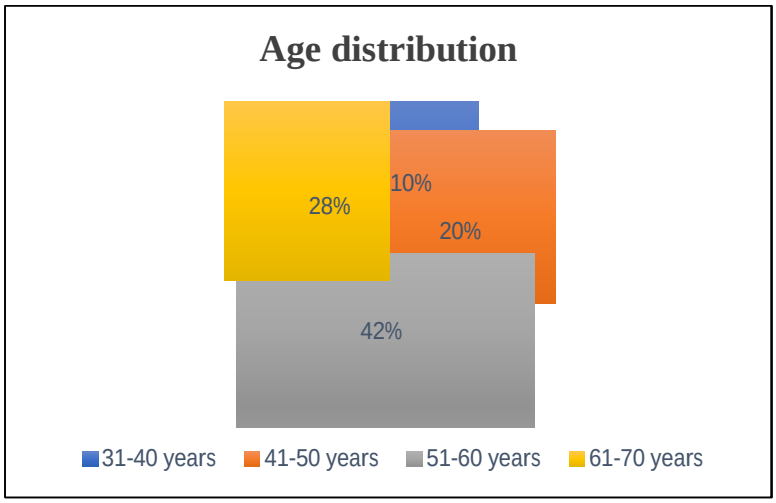


CHART I : Chart I shows age distribution of population included in our study

TABLE III: Demographics of the population based on Gender

GENDER	NUMBER OF CASES	PERCENTAGE
Male	23	57.5%
Female	17	42.5%

Table III shows sex distribution of the population included under our study. Majority(57.5%) of the population with oral squamous cell carcinoma were males.

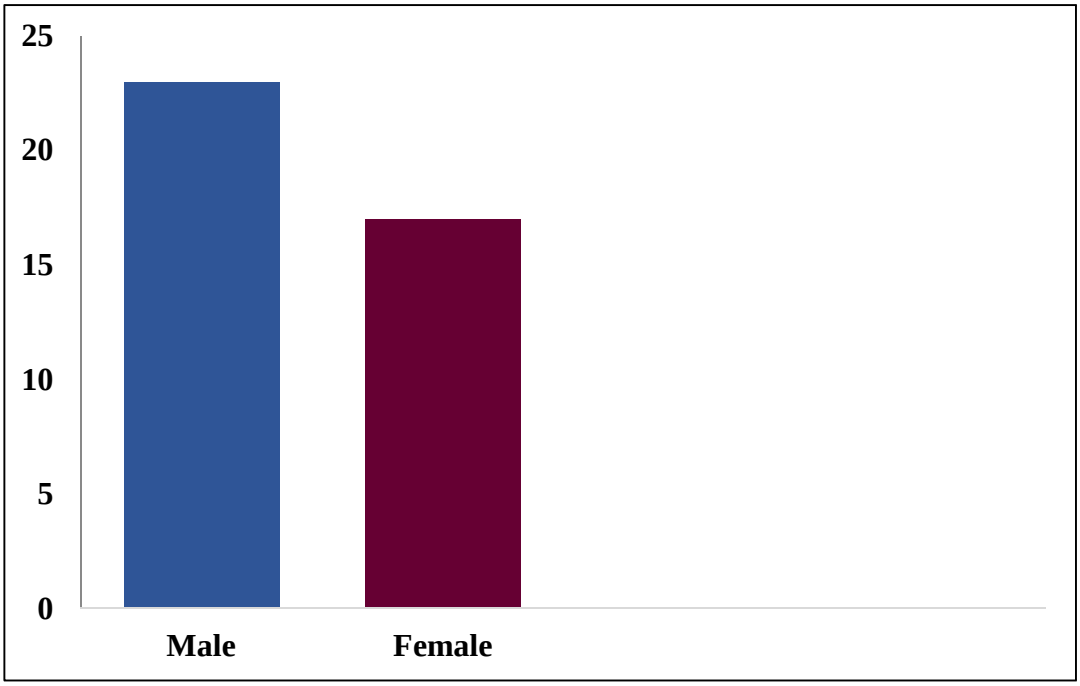


CHART II : Chart II shows gender distribution of patients with oral squamous cell carcinoma in our study

Table IV: Clinical and Histopathological characteristics

S.no	Age	Sex	Site	Grade	Nodal status
1.	60	F	Tongue	PD	+
2.	63	M	Tongue	MD	+
3.	40	F	Buccal mucosa	MD	+
4.	64	M	Buccal mucosa	PD	+
5.	60	F	Tongue	MD	+
6.	40	F	Lip	MD	+
7.	37	F	Tongue	PD	+
8.	52	M	Soft palate	MD	-
9.	56	M	Tongue	MD	+
10.	53	M	Tongue	WD	-
11.	52	M	Tonsillar fossa	MD	-
12.	60	M	Soft palate	MD	-
13.	45	F	Buccal mucosa	WD	-
14.	56	M	Buccal mucosa	MD	-
15.	68	M	Tongue	WD	-
16.	55	F	Buccal mucosa	MD	-
17.	50	M	Soft palate	MD	-
18.	65	F	Tongue	WD	-
19.	65	F	Lip	PD	+
20.	70	M	Tonsillar fossa	WD	-
21.	70	F	Retromolar trigone	WD	+
22.	58	M	Buccal mucosa	MD	-
23.	50	F	Alveolar margin	WD	-

24.	49	M	Retromolar trigone	WD	-
25.	44	M	Tongue	MD	+
26.	56	M	Tonsillar fossa	MD	-
27.	62	M	Tonsillar fossa	MD	+
28.	60	M	Retromolar trigone	MD	-
29.	43	F	Alveolar margin	WD	-
30.	65	M	Tonsillar fossa	MD	-
31.	45	M	Tonsillar fossa	MD	-
32.	55	M	Buccal mucosa	WD	-
33.	60	F	Floor of mouth	MD	+
34.	61	F	Lip	PD	+
35.	40	M	Tongue	MD	+
36.	60	F	Lip	WD	+
37.	60	M	Retromolar trigone	MD	+
38.	48	M	Tongue	MD	+
39.	63	F	Tonsillar fossa	MD	+
40.	60	F	Lip	WD	+

WD - Well differentiated, MD – Moderately differentiated, PD - Poorly differentiated, - Absent, + Present.

Table IV shows clinical and histopathological characteristics of individual patients.

TABLE V: Distribution of tumour within the oral cavity

S.NO	SITE OF THE TUMOUR	NUMBER OF PATIENTS	PERCENTAGE
1.	Tongue	11	27.5%
2.	Buccal mucosa	7	17.5%
3.	Tonsillar fossa	7	17.5%
4.	Lip	5	12.5%
5.	Retromolar trigone	4	10%
6.	Soft palate	3	7.5%
7.	Alveolar margin	2	5%
8.	Floor of mouth	1	2.5%

Table V shows the distribution of tumour within oral cavity. Of the specimens received 11 patients had squamous cell carcinoma in tongue, 7 each from buccal mucosa and tonsillar fossa, 5 from lip, 4 from retromolar trigone , 3 from soft palate, 2 from alveolar margin and 1 from floor of mouth.

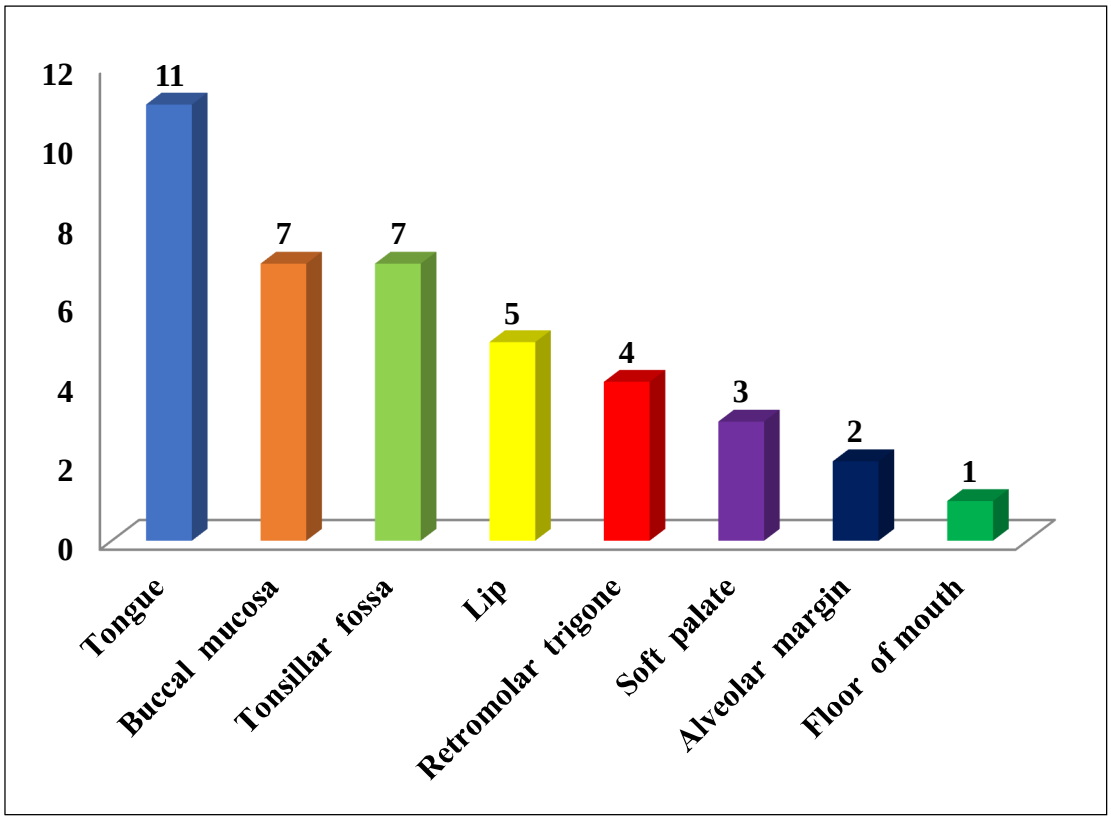


CHART III: Chart III demonstrating the distribution of tumour within oral cavity.

TABLE VI: Histopathological grade of the tumour

GRADE	NUMBER OF CASES	PERCENTAGE
Well differentiated	12	30%
Moderately differentiated	23	57.5%
Poorly differentiated	5	12.5%

Table VI shows the histopathological grade of oral squamous cell carcinoma in the patients included in the study. Majority of population had moderately differentiated grade of Squamous cell carcinoma.

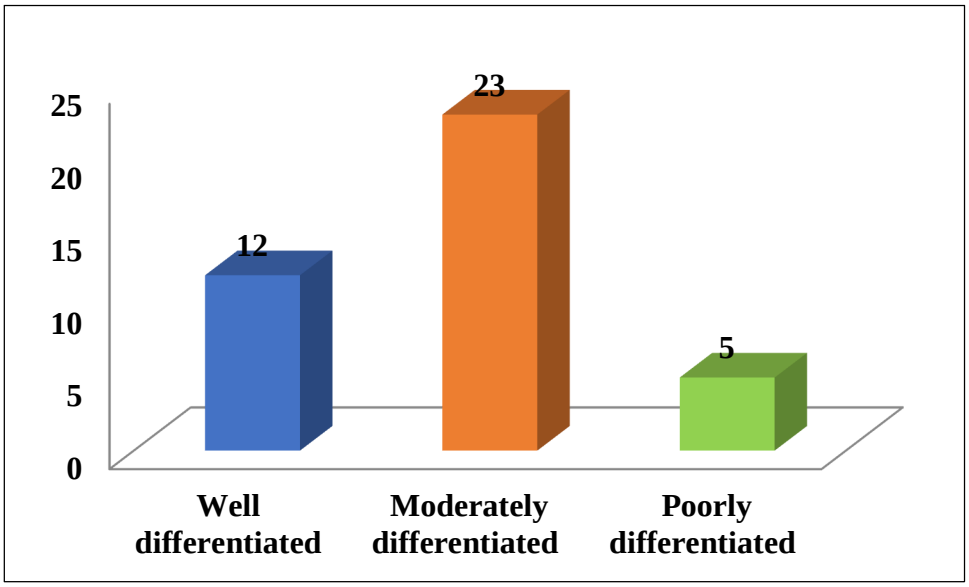


CHART IV: Chart IV shows the histopathological grade of oral squamous cell carcinoma of the population of our study.

PATTERN OF IMMUNOSTAINING OF MOLECULAR MARKERS:

Immunostaining of molecular markers E-cadherin and Vimentin were done in our study. Staining for E-cadherin demonstrated membrane pattern of staining. Staining of Vimentin demonstrated cytoplasmic pattern of staining.

TABLE VII : Scoring of molecular marker E-cadherin & Vimentin in patients with oral squamous cell carcinoma

S.NO	HPE NO	E- CADHERIN				VIMENTIN			
		PS	IS	TS	IR	PS	IS	TS	IR
1.	1688/17	2	2	4	Low	3	3	9	High
2.	1778/17	3	3	9	High	3	3	6	High
3.	2107/17	3	3	9	High	3	3	9	High
4.	2848/17	2	2	4	Low	3	3	9	High
5.	3346/17	2	3	6	High	3	3	9	High
6.	3364/17	4	3	12	High	3	3	9	High
7.	3385/17	2	2	4	Low	4	3	12	High
8.	3397/17	4	3	12	High	1	2	2	Low
9.	3423/17	3	3	9	High	3	3	9	High
10.	3591/17	4	3	12	High	1	2	2	Low

11.	3693/17	4	3	12	High	1	2	2	Low
12.	3694/17	4	3	12	High	1	2	2	Low
13.	3695/17	4	3	12	High	1	2	2	Low
14.	3722/17	3	3	9	High	2	2	4	Low
15.	3734/17	3	3	9	High	0	0	0	Neg
16.	3736/17	4	3	12	High	3	2	6	High
17.	4107/17	4	1	4	Low	2	3	6	High
18.	4139/17	4	3	12	High	2	2	4	Low
19.	4141/17	3	2	6	High	3	3	9	High
20.	4265/17	4	2	8	High	0	0	0	Neg
21.	1962/18	4	3	12	High	2	3	6	High
22.	1966/18	3	3	9	High	1	2	2	Low
23.	1973/18	4	3	12	High	2	2	4	Low
24.	1991/18	4	3	12	High	0	0	0	Neg
25.	1993/18	4	3	12	High	2	2	4	Low
26.	2035/18	3	3	9	High	2	3	6	High
27.	2042/18	4	3	12	High	1	2	2	Low
28.	2073/18	4	3	12	High	1	2	2	Low
29.	2076/18	3	3	9	High	1	2	2	Low
30.	2080/18	3	3	9	High	2	2	4	Low
31.	2081/18	3	3	9	High	1	2	2	Low

32.	2083/18	4	3	12	High	1	2	2	Low
33.	2131/18	3	3	9	High	3	3	9	High
34.	2171/18	2	2	4	Low	3	3	9	High
35.	2348/18	4	3	12	High	3	2	6	High
36.	2838/18	4	3	12	High	3	2	6	High
37.	2888/18	3	3	9	High	2	3	6	High
38.	2980/18	2	3	6	High	2	3	6	High
39.	2982/18	3	3	9	High	2	3	6	High
40.	3038/18	4	3	12	High	3	3	9	High

PS – Proportion score (Scoring 0 – Negative, 1 - < 10% of the cells taken up the stain, 2 – 10-20% of the cells, 3- 20-50% of the cells, 4- >50% of the cells)

IS – Intensity score(Scoring 0 – Negative, 1 – Weak, 2 – Moderate, 3 – Strong)

TS – Total score(ranges from 0 to 12)

IR – Immunoreactivity(Scoring 0 – Negative, 1-4 – Low, >4 – High)

Table VII shows immunoreactivity of E-cadherin and vimentin immunohistochemical marker in malignant cells.

TABLE VIII: Immunoreactivity of E-cadherin in patients with oral squamous cell carcinoma

IMMUNOREACTIVITY	NUMBER OF CASES	PERCENTAGE
Negative (0)	0	0
Low (1 – 4)	5	12.5%
High (>4)	35	87.5%

Table VIII: This Table shows that most of the cases of oral squamous cell carcinoma with E-Cadherin expression were of high immunoreactivity score.

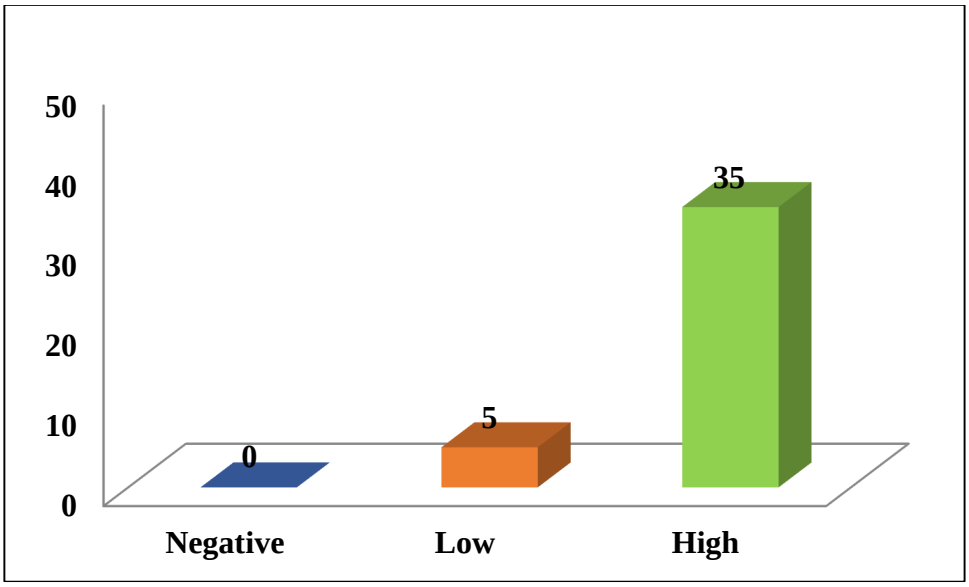


CHART V: The Chart shows the immunoreactivity of E-Cadherin in oral squamous cell carcinoma.

TABLE IX: Immunoreactivity of Vimentin in patients with oral squamous cell carcinoma

IMMUNOREACTIVITY	NUMBER OF CASES	PERCENTAGE
Negative (0)	3	7.5
Low (1 – 4)	16	40.0
High (>4)	21	52.5

Table IX shows vimentin expression which was high in 52.5% of the cases and low in 40% of the cases with oral squamous cell carcinoma.

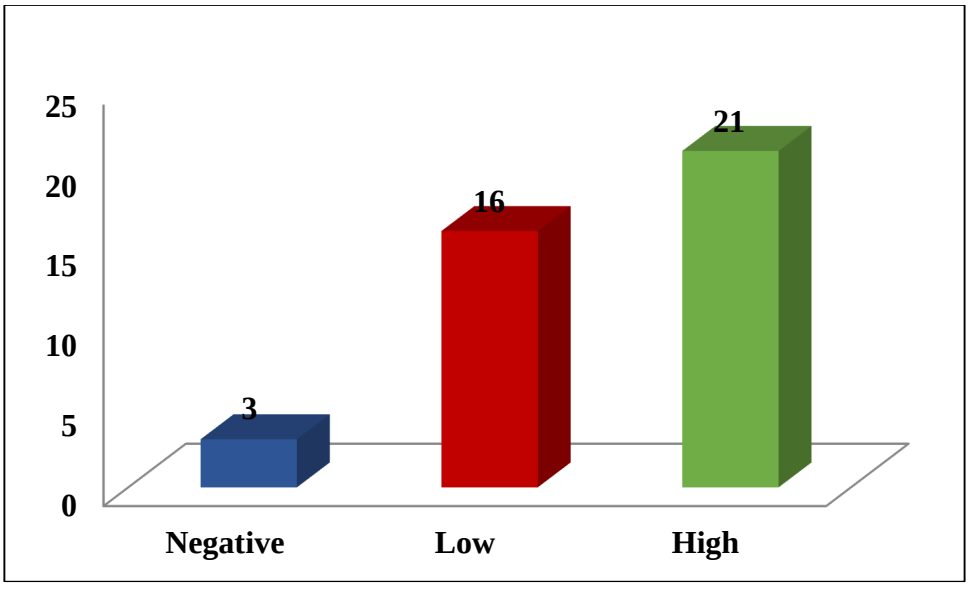


CHART VI: The Chart demonstrates the immunoreactivity of Vimentin in tumour included in our study.

TABLE X: Expression of E-cadherin in patients with oral squamous cell carcinoma in correlation with histopathological grade of tumour.

Histopathological Grade	Immunoreactivity			P Value
	Negative	Low	High	
Well differentiated	-	0(0.0%)	12(100.0%)	0.000*
Moderately differentiated	-	1(4.3%)	22(95.7%)	
Poorly differentiated	-	4(80.0%)	1(20.0%)	

*-statistically significant (P<0.05)

Table X shows that the E-Cadherin expression was statistically significant with histopathological grade of the tumour

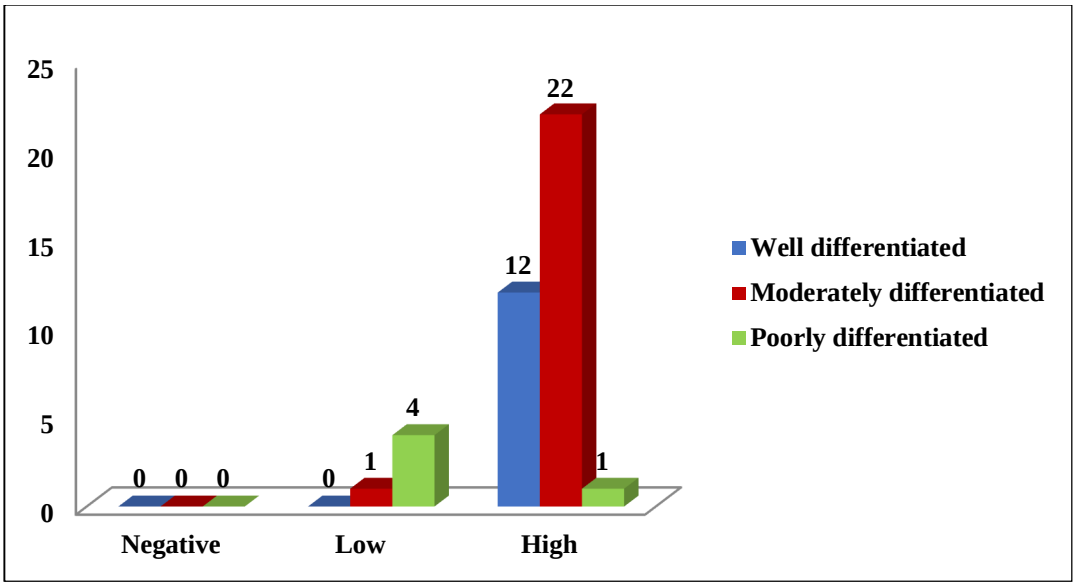


CHART VII: Chart showing E-Cadherin expression in relation to histopathological grade of tumour

TABLE XI: Expression of E-cadherin in patients with oral squamous cell carcinoma in relation to nodal metastasis

NODAL METASTASIS	IMMUNOREACTIVITY			P VALUE
	NEGATIVE	LOW	HIGH	
Present	-	4(20.0%)	16(80.0%)	.154
Absent	-	1(5.0%)	19(95.0%)	

Table XI shows that there was no significant relationship between E-Cadherin expression and nodal metastasis.

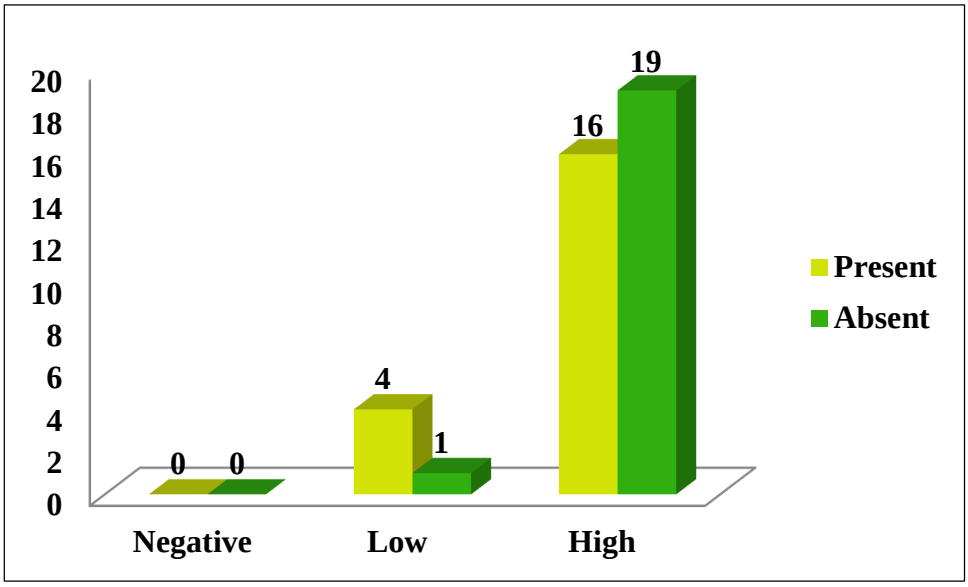


CHART VIII: This chart shows that E-Cadherin was expressed in tumour with and without nodal metastasis.

TABLE XII: Expression of Vimentin in patients with oral squamous cell carcinoma with correlation to histopathological grade of tumour

HISTOPATHOLOGICAL GRADE	IMMUNOREACTIVITY			P VALUE
	NEGATIVE	LOW	HIGH	
Well differentiated	3(25.0%)	6(50.0%)	3(25.0%)	0.010*
Moderately differentiated	0(0.0%)	10(43.5%)	13(56.5%)	
Poorly differentiated	0(0.0%)	0(0.0%)	5(100.0%)	

*-statistically significant (p<0.05)

Table XII demonstrates significant relationship between expression of vimentin and histopathological grade of tumour.

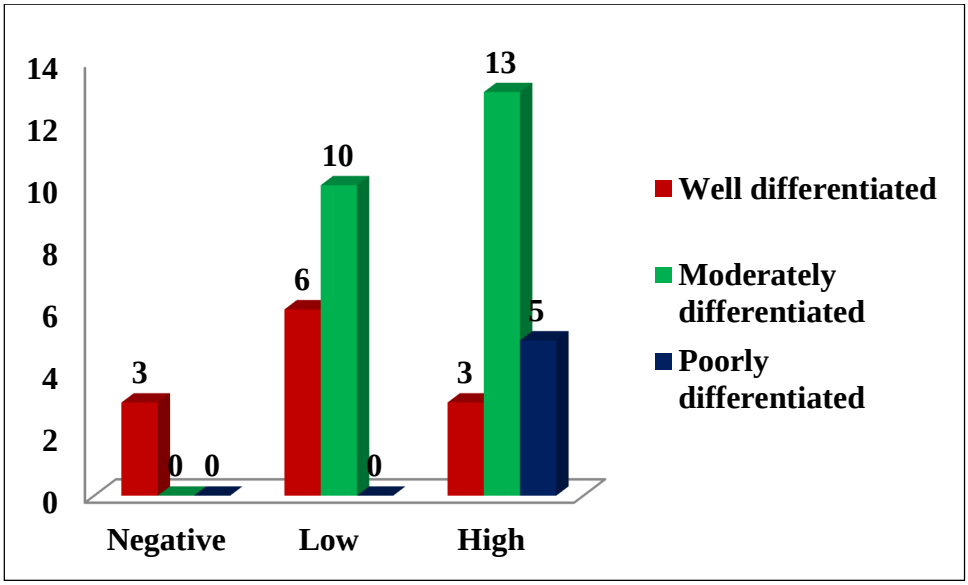


CHART IX: Chart showing Vimentin expression in relation to grade of tumour

TABLE XIII: Expression of Vimentin in patients with oral squamous cell carcinoma in relation to nodal metastasis

NODAL METASTASIS	IMMUNOREACTIVITY			P VALUE
	NEGATIVE	LOW	HIGH	
Present	0(0.0%)	2(10.0%)	18(90.0%)	.000*
Absent	3(15.0%)	14(70.0%)	3(15.0%)	

*-statistically significant (P<0.05)

Table XIII shows the Vimentin expression was statistically significant in oral squamous cell carcinoma with nodal metastasis

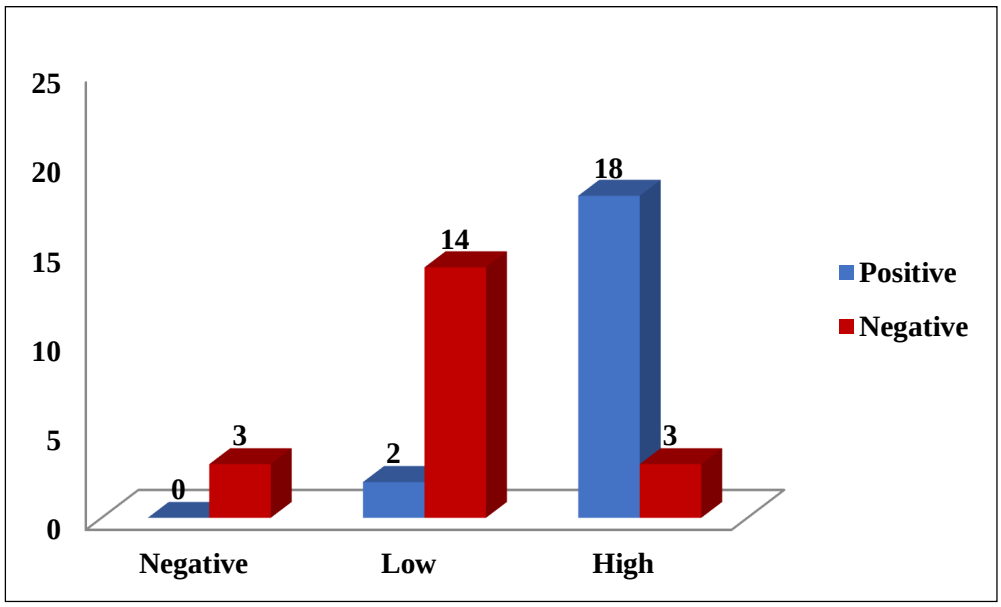


CHART X: Chart showing expression of Vimentin in tumour presented with nodal metastasis

TABLE XIV: Expression of E-Cadherin and Vimentin in relation to histopathological grade in patients with nodal metastasis

Histopathological grade	Nodal metastasis (N=20)	E-cadherin immunoreactivity			Vimentin immunoreactivity		
		Low	High	P value	Low	High	P value
Well differentiated	3	0 (0%)	3 (100%)	0.001*	0 (0%)	3 (100%)	0.477
Moderately differentiated	12	0 (0%)	12 (100%)		2 (16.7%)	10 (83.3%)	
Poorly differentiated	5	4 (80%)	1 (20%)		0 (0%)	5 (100%)	

*-statistically significant (P<0.05)

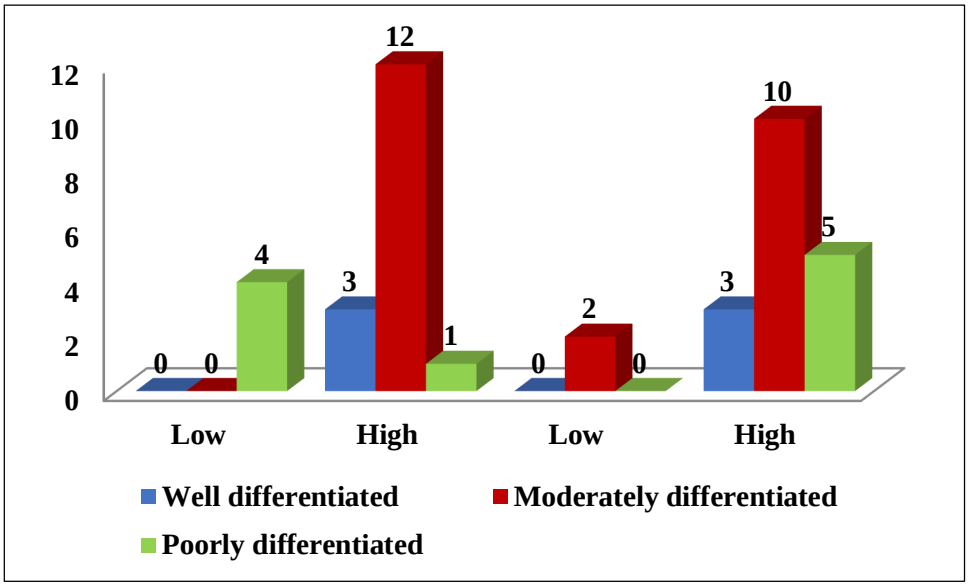
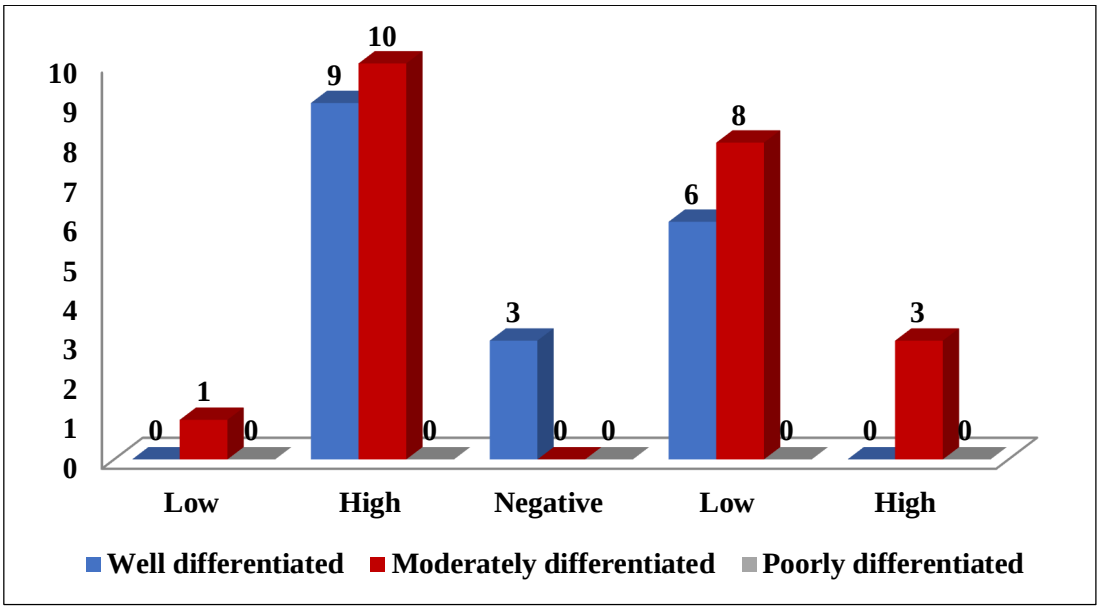


CHART XI: E-Cadherin and Vimentin expression in relation to grade in patients with nodal metastasis

Table XV: Expression of E-Cadherin and Vimentin in relation to histopathological grade in patients without nodal metastasis:

Histopathological grade	Nodal metastasis absent (N=20)	E-Cadherin immunoreactivity			Vimentin immunoreactivity			
		Low	High	P Value	Negative	Low	High	P Value
Well differentiated	**9**	0 (0%)	9 (100.0%)	0.351	3 (33.3%)	6 (66.7%)	0 (0%)	0.046*
Moderately differentiated	**11**	1 (9.1%)	10 (90.9%)		0 (0%)	8 (72.7%)	3 (27.3%)	
Poorly differentiated	**0**	-	-		-	-	-	

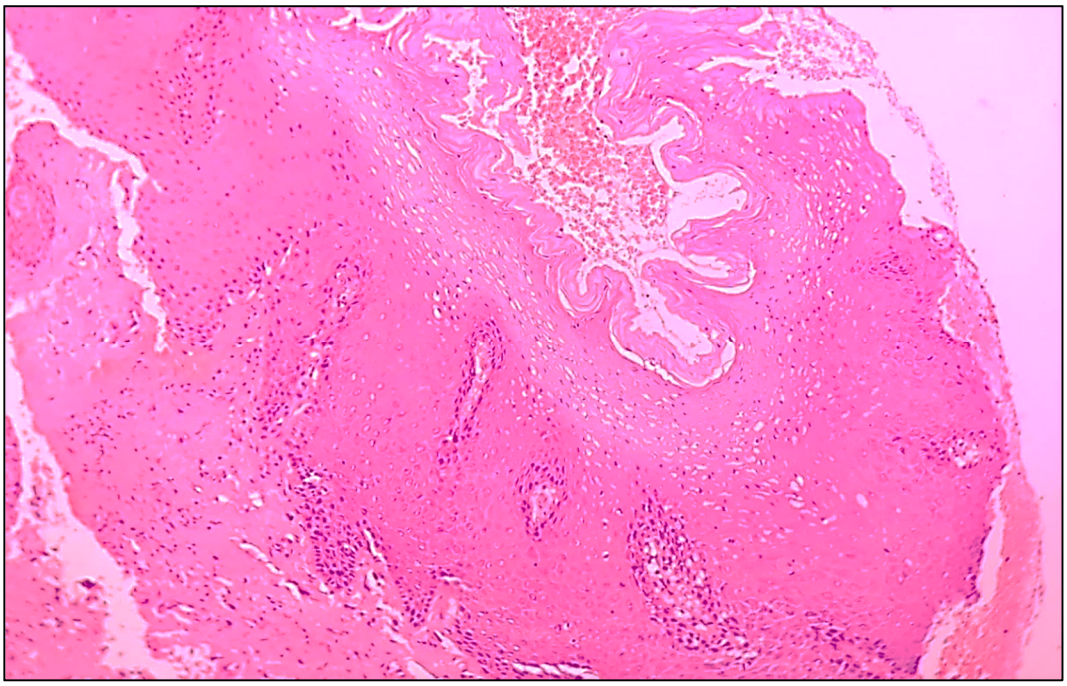
*-statistically significant (P<0.05)



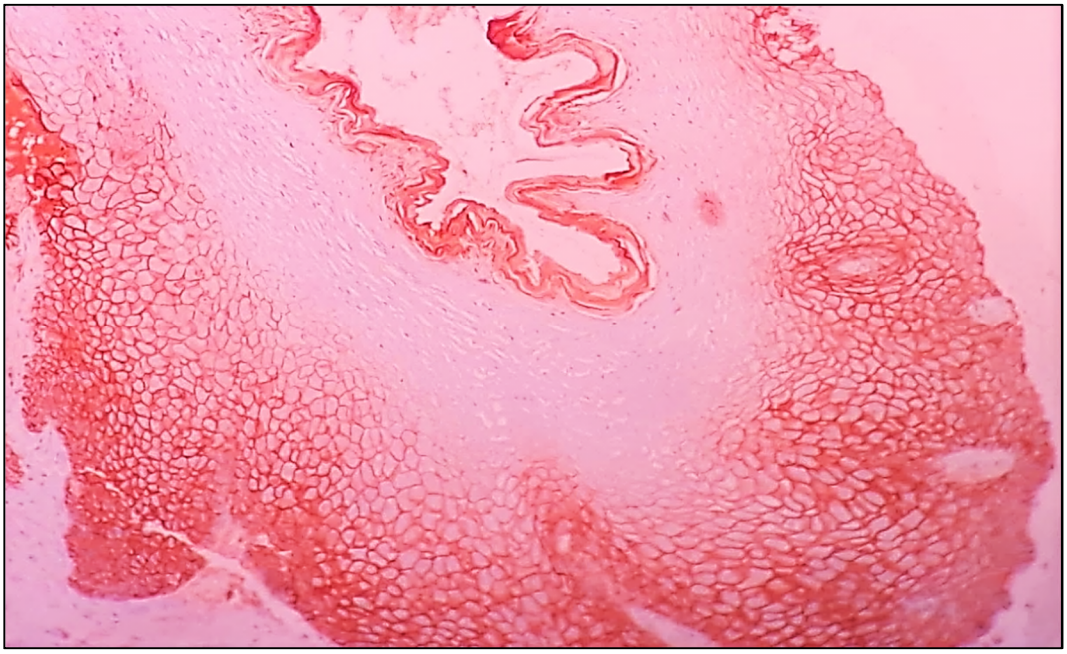
E-cadherin **Vimentin**

CHART XII: E-Cadherin and Vimentin expression in tumour in relation to histopathological grade in patients without nodal metastasis.

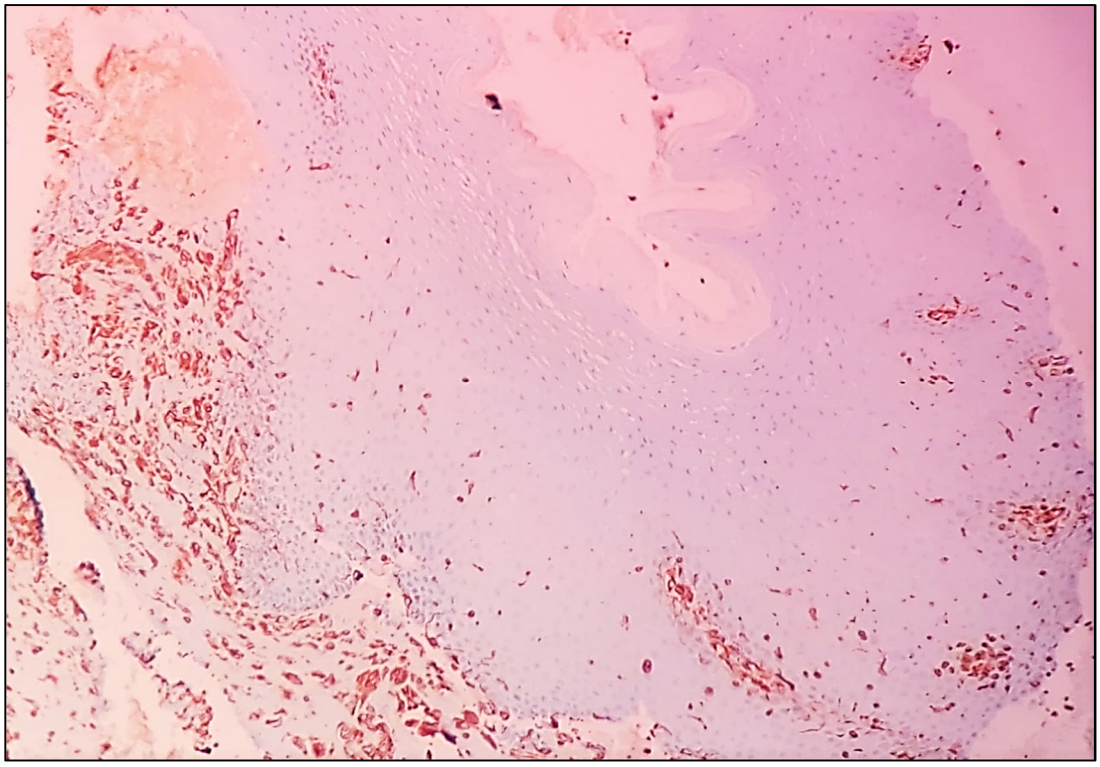
COLOUR PLATES



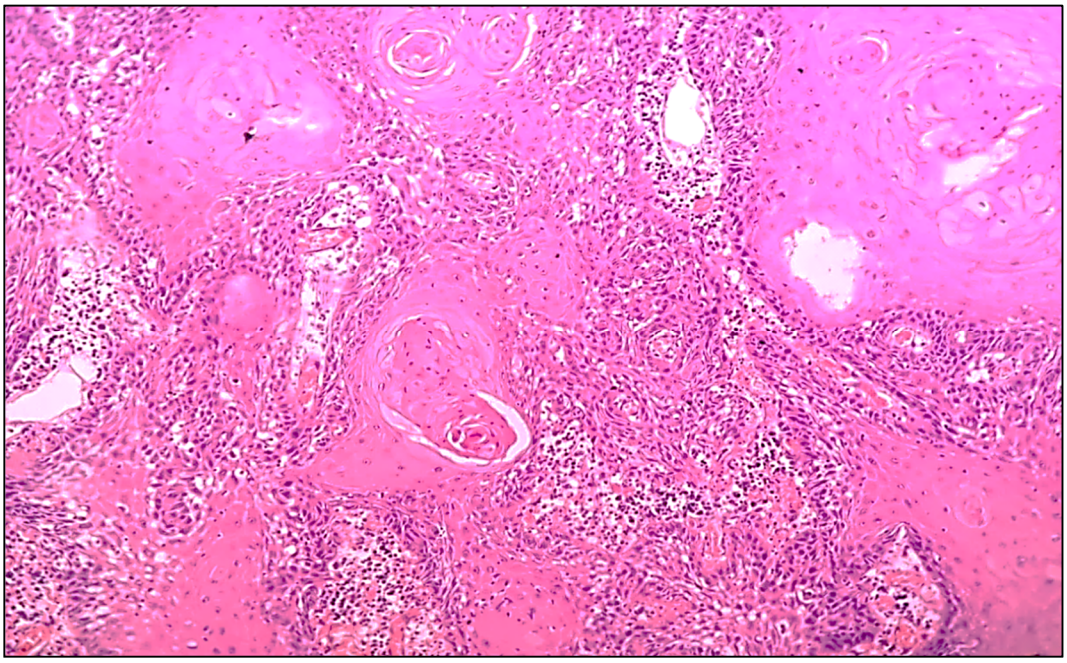
COLOUR PLATE 1: Photomicrograph of H & E stained sections of normal oral squamous epithelium (Magnification :100X)



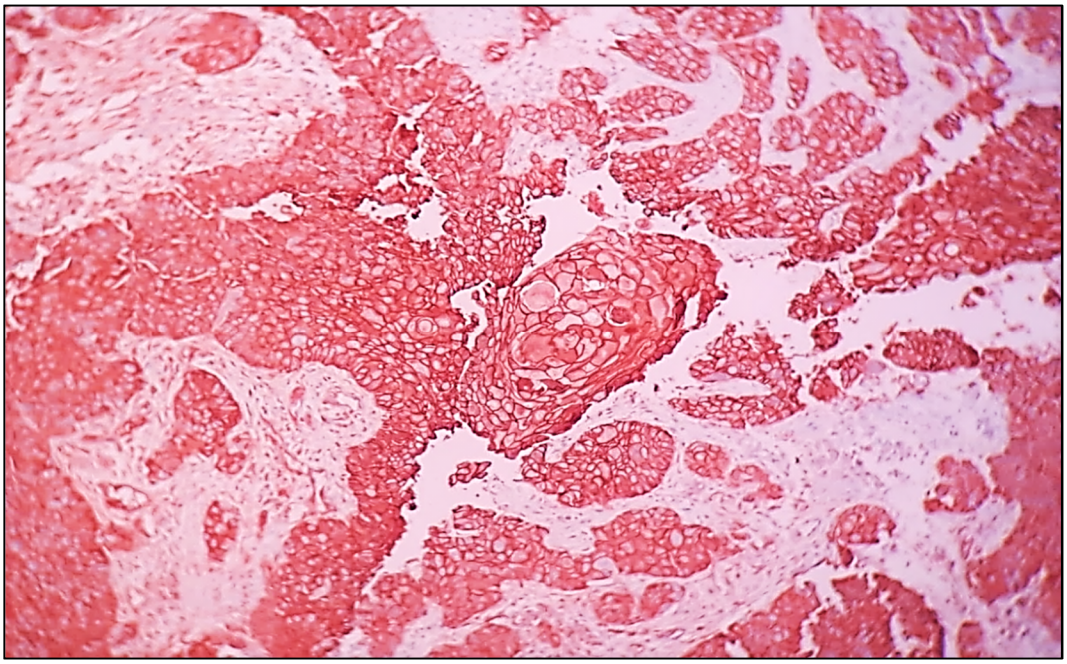
COLOUR PLATE 2: Photomicrograph of the Positive E-Cadherin immunohistochemical staining in normal oral squamous epithelium. (Magnification :100X)



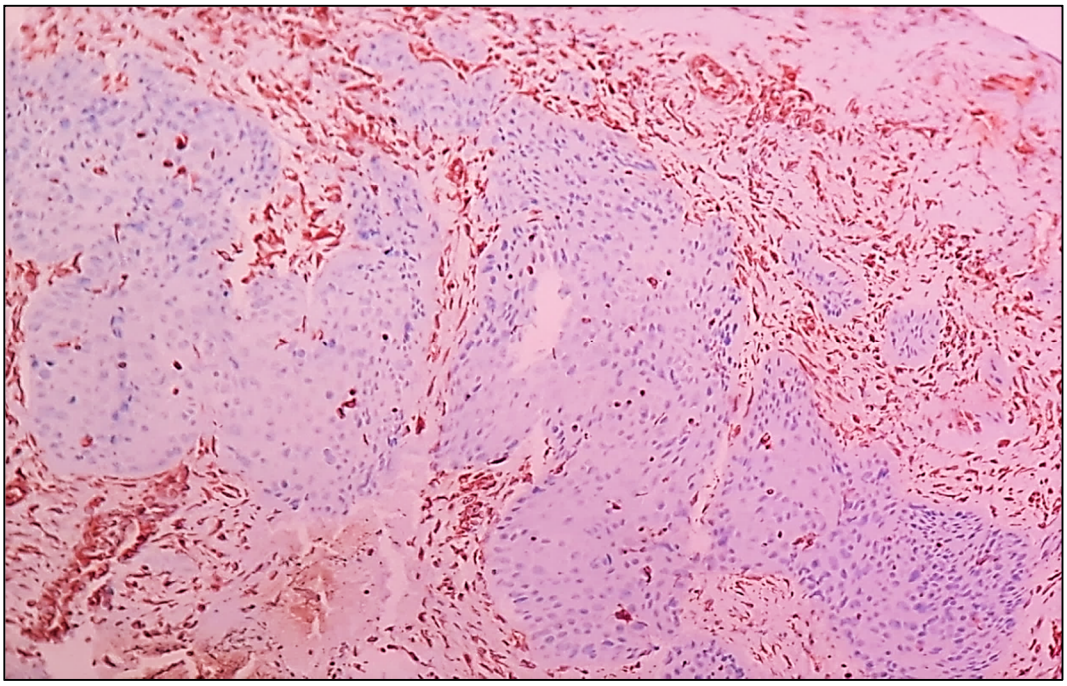
COLOUR PLATE 3: Photomicrograph of Negative Vimentin immunohistochemical staining in normal oral squamous epithelium. (Magnification : 100X)



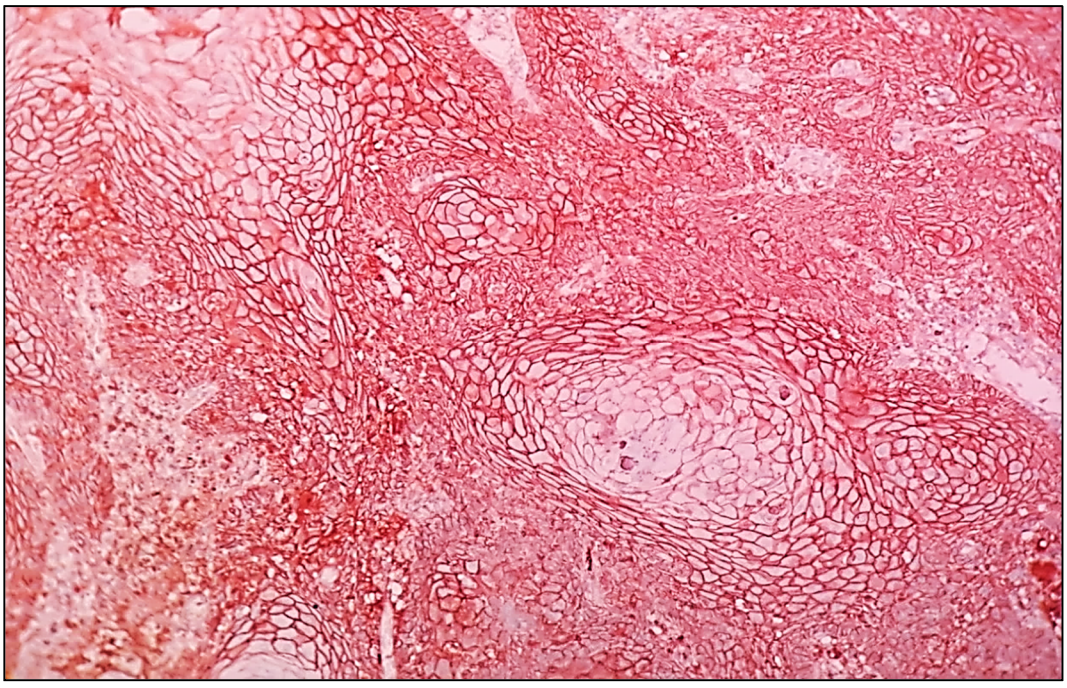
COLOUR PLATE 4 : Photomicrograph of H & E stained section of Oral Squamous cell carcinoma - Well differentiated grade (Magnification :100X)



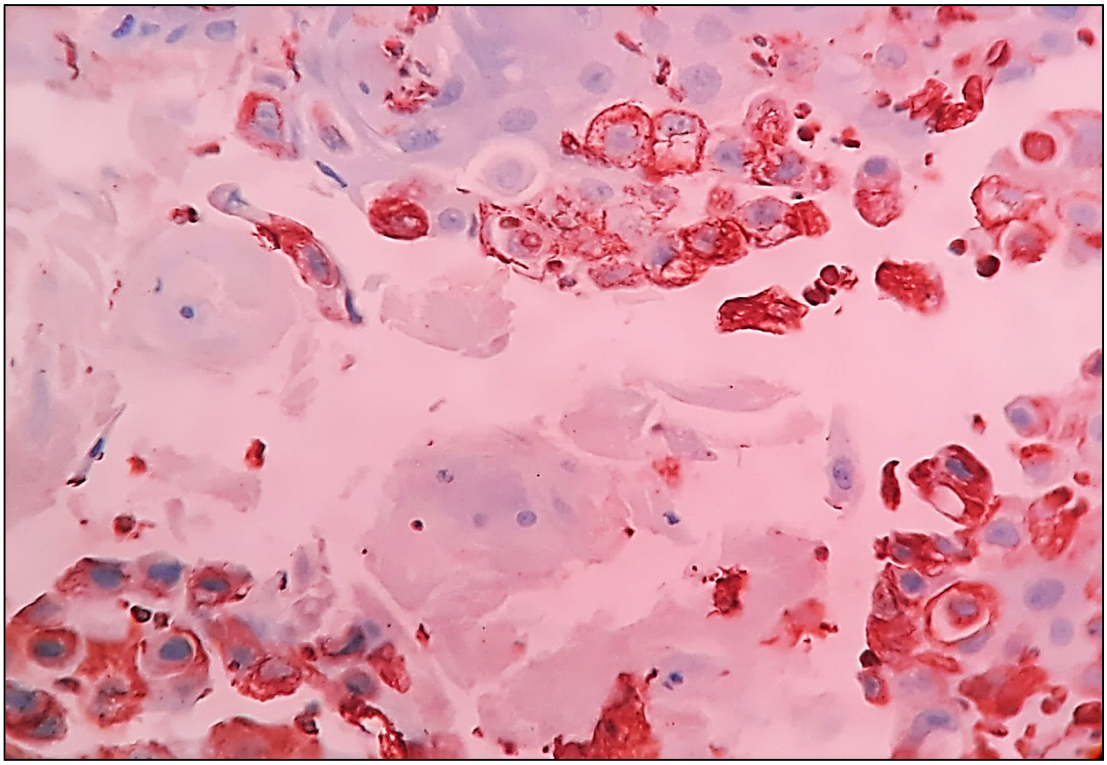
COLOUR PLATE 5: Photomicrograph of E-Cadherin positive staining showing high immunoreactive score in Well differentiated grade without nodal metastasis(Magnification :100 X)



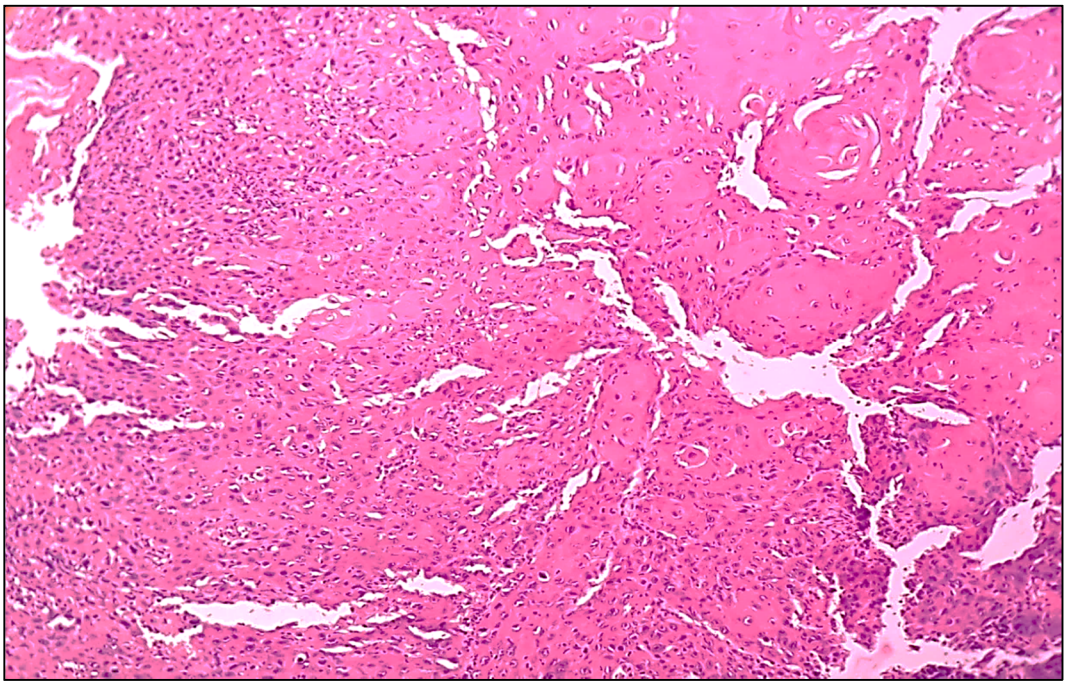
COLOUR PLATE 6 : Photomicrograph of Vimentin staining showing negative immunoreactivity in Well differentiated grade without nodal metastasis.(Magnification :100X)



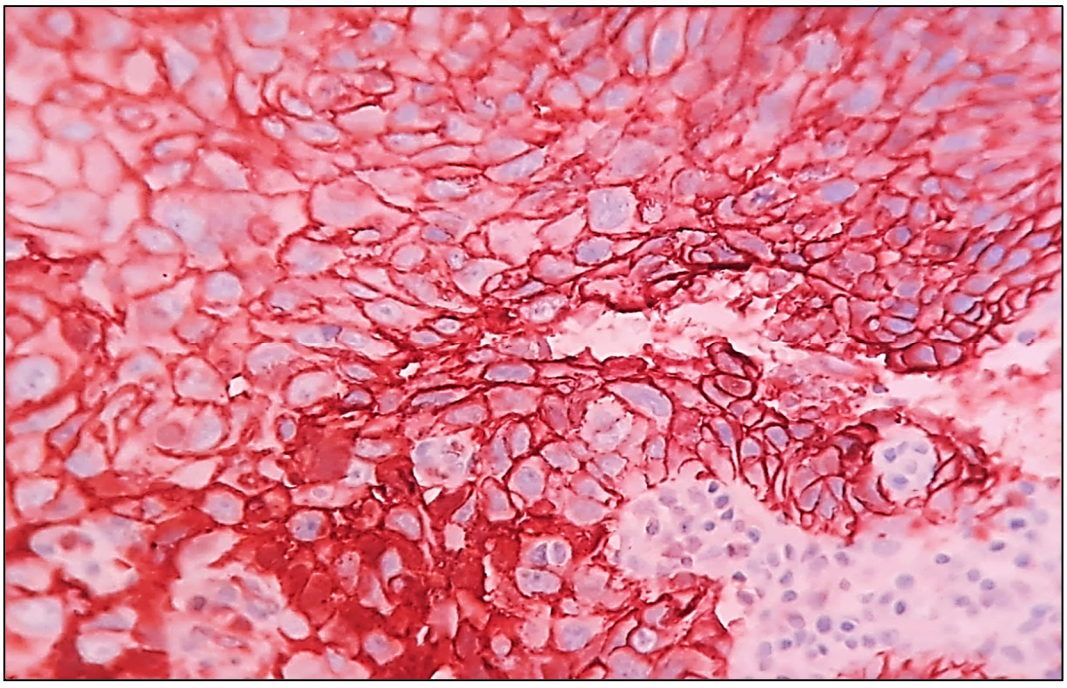
COLOUR PLATE 7: Photomicrograph of E-cadherin staining showing high immunoreactivity in Well differentiated grade with nodal metastasis.(Magnification :100X**)**



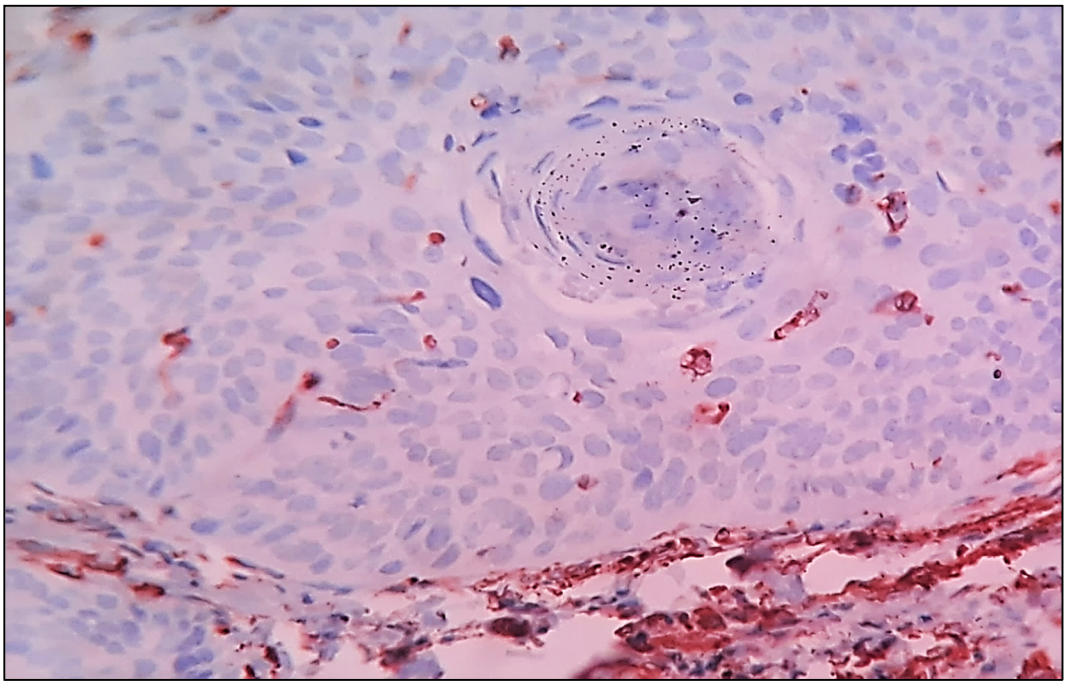
COLOUR PLATE 8: Photomicrograph of Vimentin staining with high immunoreactivity in Well differentiated grade with nodal metastasis.(Magnification :400X**)**



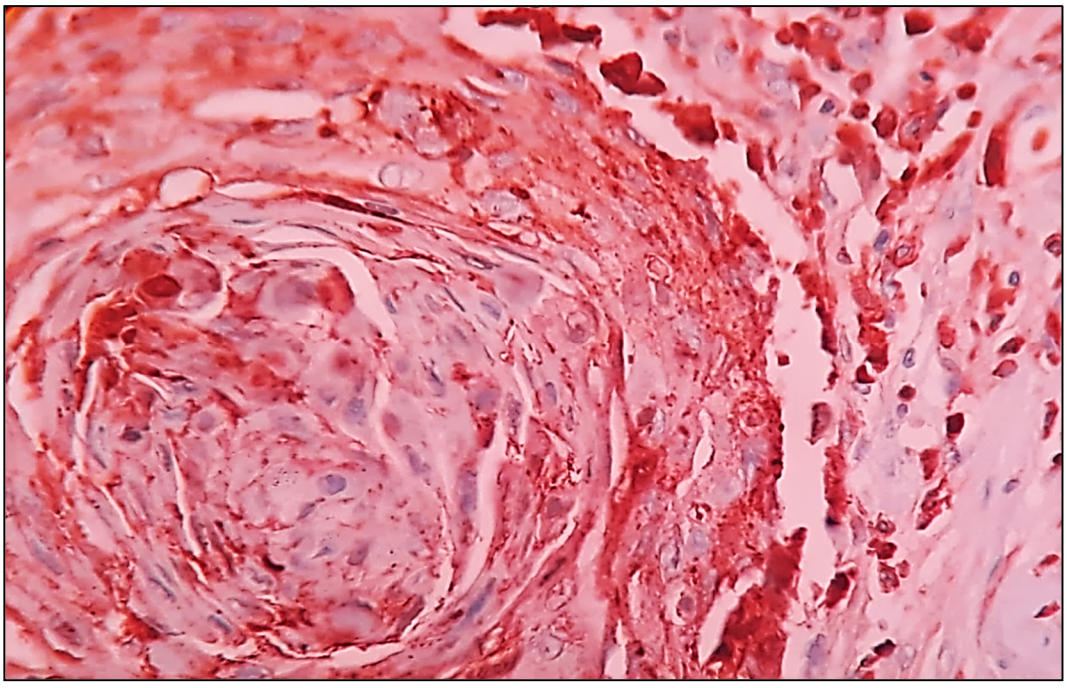
COLOUR PLATE 9: Photomicrograph of H &E stained sections of Moderately differentiated grade of Oral squamous cell carcinoma.(Magnification :100X)



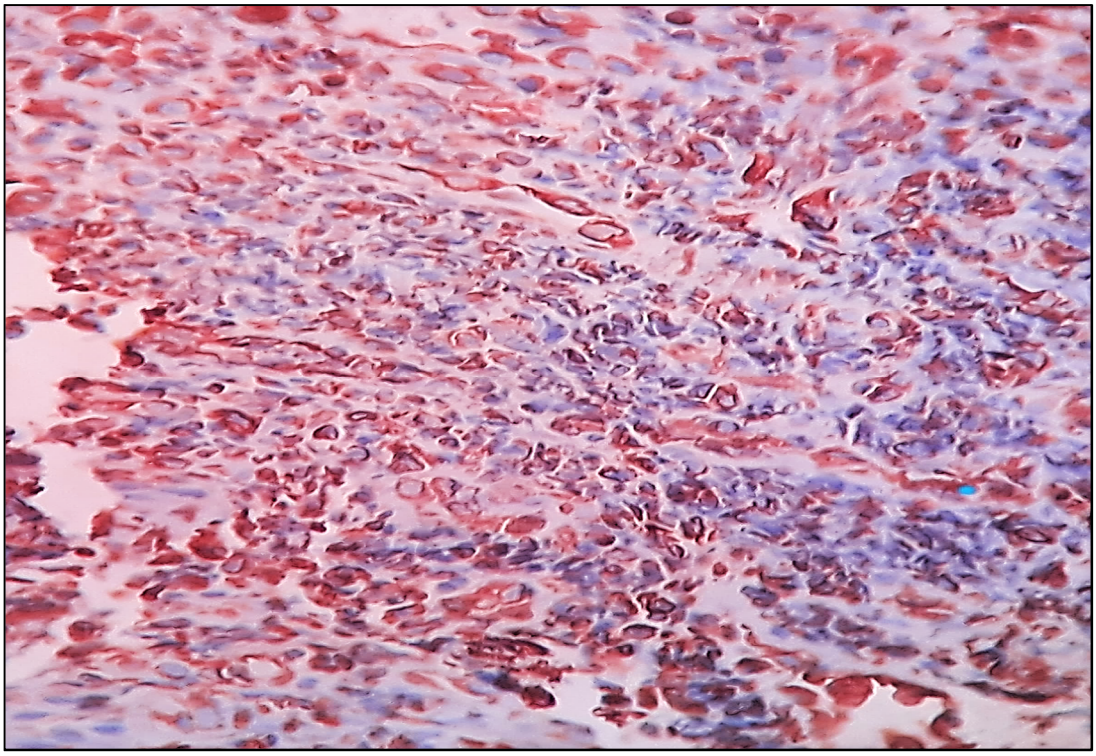
COLOUR PLATE 10: Photomicrograph of E-Cadherin staining with high immunoreactivity in Moderately differentiated grade without nodal metastasis. (Magnification: 400 X**)**



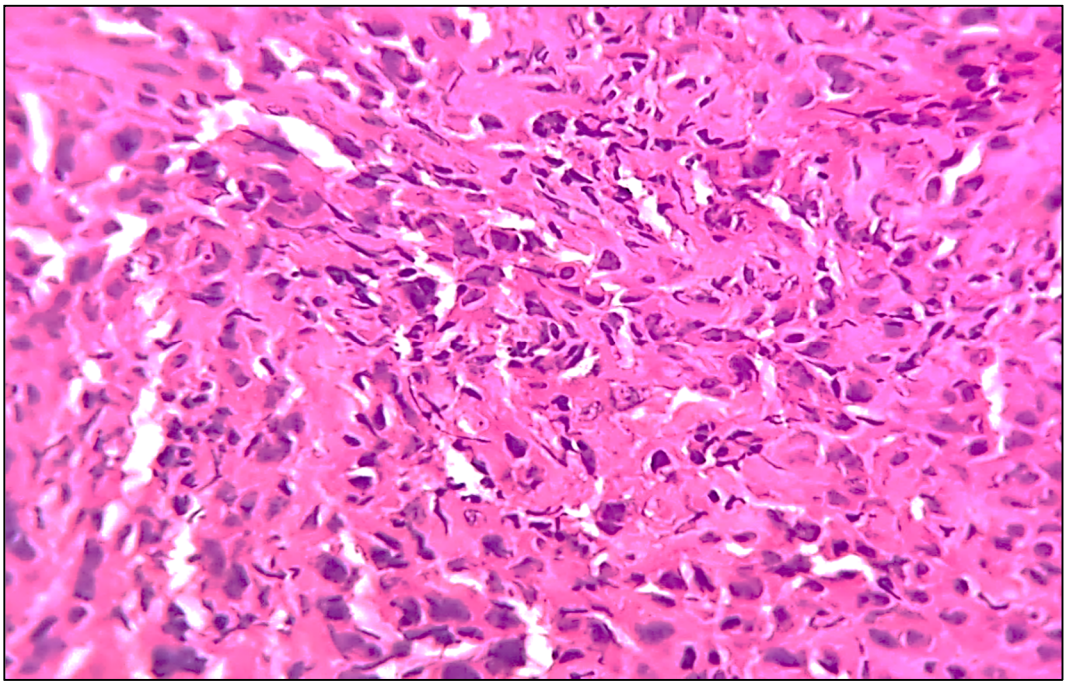
COLOUR PLATE 11: Photomicrograph of Vimentin staining with low immunoreactivity in Moderately differentiated grade without nodal metastasis.(Magnification: 400 X)



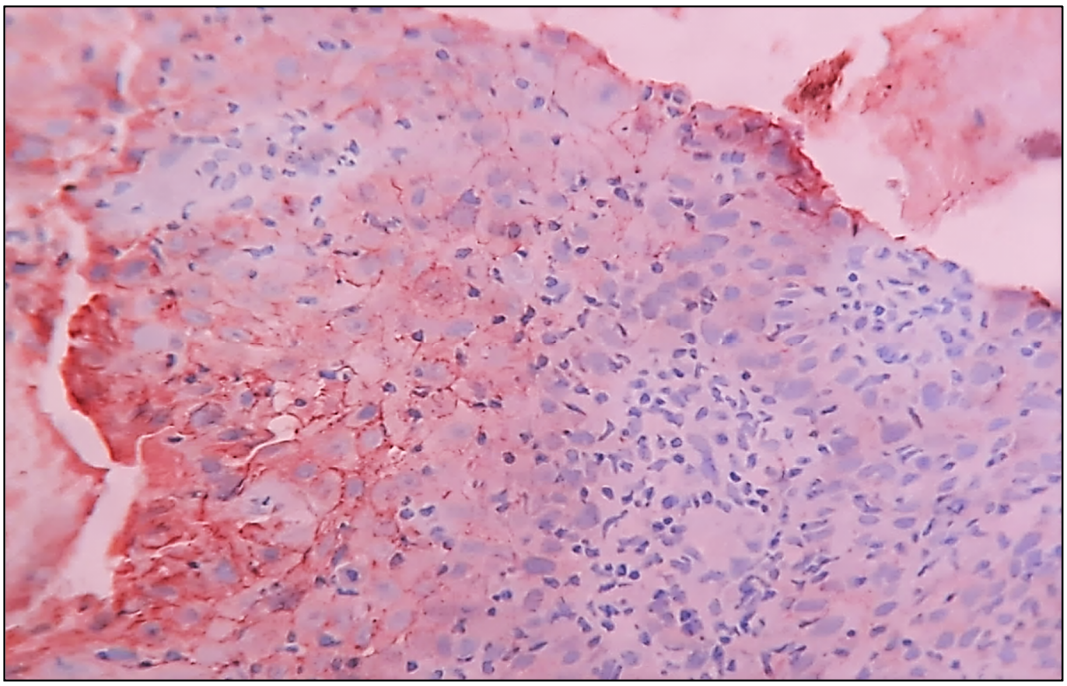
COLOUR PLATE 12 : Photomicrograph of E-Cadherin staining with high immunoreactivity in Moderately differentiated grade with nodal metastasis.(Magnification :400X**)**



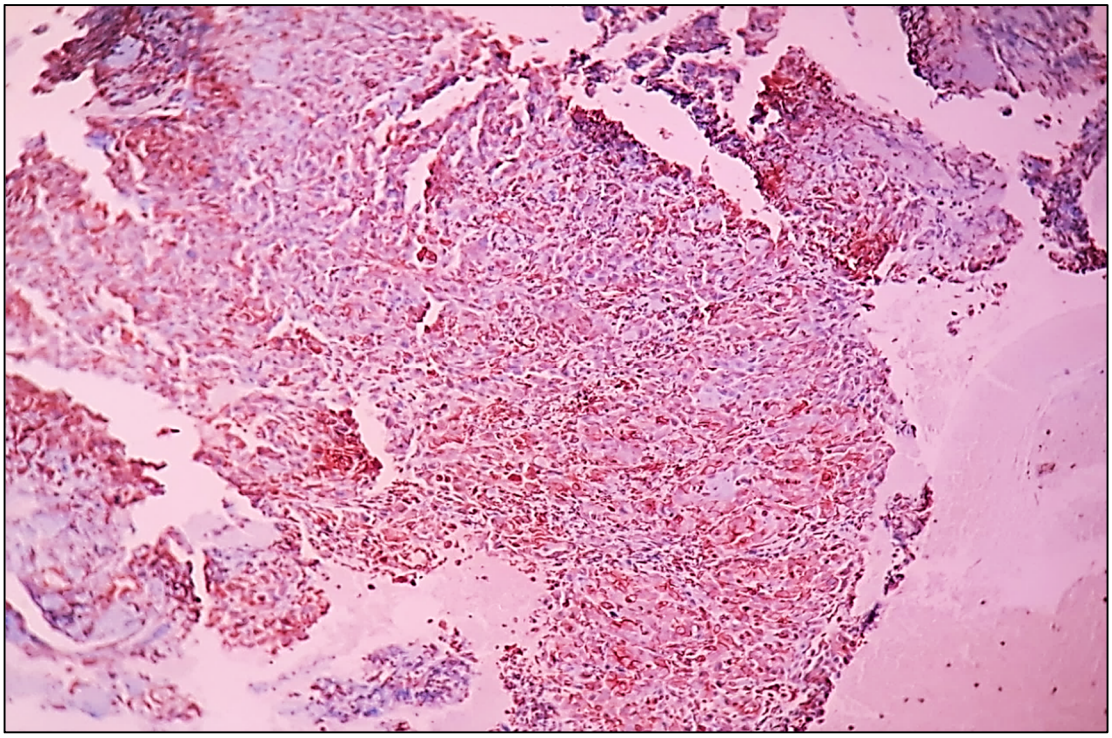
COLOUR PLATE 13: Photomicrograph of Vimentin staining showing high immunoreactive score in Moderately differentiated grade with nodal metastasis. (Magnification :400X**)**



COLOUR PLATE 14: Photomicrograph of H&E stained sections of Poorly differentiated Oral squamous cell carcinoma (Magnification :400X**)**



COLOUR PLATE 15: Photomicrograph of E-Cadherin stained sections with low immunoreactivity in Poorly differentiated carcinoma with nodal metastasis (Magnification: 400X**)**



COLOUR PLATE 16: Photomicrograph of Vimentin staining in Poorly differentiated grade with nodal metastasis with high immunoreactive score.(Magnification :100X)

DISCUSSION

The majority of Oral cancers are Squamous Cell Carcinoma, and many studies have been done on them with objective of understanding diagnosis, prognosis, and management of this entity.[102-106] Epithelial Mesenchymal transition is a biologic process in which cohesive polarized epithelial cells transform into mesenchymal like cells that exhibit no polarization and high mobility. This process is associated with cancer metastasis and invasion, that correlates with poor prognosis. So, We have studied the epithelial marker E-cadherin and mesenchymal marker Vimentin in tumour differentiation and nodal metastasis.

AGE OF OCCURRENCE:

The mean age of the patients included in our study was 55.50 years (range 51-60 years). Most affected age group in a study done by Parul et al were above 50 years old.[107] Most of the patients diagnosed in a study done by Alessandro et al were between 51 and 70 years(53.9%)[108]

GENDER DISTRIBUTION:

Majority of population suffering from Oral squamous cell carcinoma included in our study were males (57.5%). More Male cases

have also been reported in study done by Xinjia et al, which was about 70%.[109] The Incidence of Oral Cancer in Males was 53.2% and females was 46.8% in study done by Abdulrahman et al.[110]

SITE DISTRIBUTION:

In a study done by Susam et al, Tongue (62.1%) was the most common site of squamous cell carcinoma.[111] Site distribution of Oral squamous cell carcinoma are tongue - 37% followed by alveolar mucosa/gingiva - 20% and floor of mouth -19% was the result of study by Fabio et al.[112]

The Commonest tumour site in our study group was Tongue(27.5%), followed by buccal mucosa(17.5%) and tonsillar fossa(17.5%).

HISTOPATHOLOGICAL GRADE:

Most of the cases of Oral squamous cell carcinoma were Moderately differentiated grade(57.5%)in our study.

In Study done by Doshi et al, of the 57 cases of Oral Squamous cell Carcinoma, the well differentiated, moderately differentiated and Poorly differentiated were 52.6%, 42.1%,3.5% respectively.[113]

Yusheng Jin reported that moderately differentiated formed the major bulk of their study.[114]

CORRELATION OF E-CADHERIN IN ORAL SCC WITH HISTOPATHOLOGICAL GRADE:

In our study decreased E-Cadherin (epithelial marker) and increased Vimentin(mesenchymal marker) that correlated with disease severity was observed. E-Cadherin showed high immunoreactivity score (strong membranous staining) in well(100%) and moderately differentiated grades (95.7%) and low immunoreactivity score (weak cytoplasmic staining) in poorly differentiated grade (80%). Reduced expression of e cadherin is favouring towards poorly differentiated grade and invasion. Thus, E-Cadherin expression has statistically significant association (p value : 0.000) with histopathological grade.

A study by Mehendiratta et al observed that absence of E-Cadherin staining in 0%,10%,30% of well, moderate and poorly differentiated oral squamous cell carcinoma, respectively.[115] Kaur et al studied E-Cadherin expression in histological grades of Oral squamous cell carcinoma and reported strong expression in 90% well differentiated, 92.9% of moderately differentiated and 15.4% of poorly differentiated

carcinoma as compared to weak and homogenous staining in 10%,7.1% and 69.2% respectively.[116] A reduced E-Cadherin expression was noted in oral SCC with increase in histological grade in study done by Punnya et al.[117]

EXPRESSION OF E-CADHERIN IN RELATION TO NODAL METASTASIS:

Bagutti et al have shown that poorly differentiated tumours showed reduced expression of E-Cadherin and these tumour cells acquire invasive phenotype.[118] Similar were the results of Tanaka et al, who noticed a significant relationship between reduced E-Cadherin and invasiveness of oral SCC.[119]

Hubner et al found that downregulation of E-Cadherin expression in cancer cell is associated with occult metastasis in oral cavity and oropharyngeal squamous cell carcinomas. These findings support the function of E-Cadherin in tumour suppression and lymphogenous metastasis in vivo.[120]

Study done by Sun et al reported that loss of E-Cadherin was associated with infiltrative growth pattern and nodal metastasis, but not distant site metastasis.[121] Reduced staining intensity of E-cadherin has

been correlated with presence of nodal metastasis in study of 101 patients with supraglottic laryngeal carcinoma by Rodrigo et al.[122]

However, Bulkholm et al reported that there was no significant difference between E-Cadherin expression in cases with and without regional nodal metastasis in human breast carcinoma and that variation in the expression of E-Cadherin vary from tumour to tumour.[123] Cavallaro and Christofori have stated that loss of E-cadherin is a hallmark of metastatic carcinoma.[124]

In our study E-Cadherin expression was not statistically significant in oral SCC patients with nodal metastasis. (p value: 0.154)

EXPRESSION OF VIMENTIN IN RELATION TO GRADE :

Expression of Vimentin was low/negative in 75% of well differentiated cases. But majority of moderately differentiated(56.5%) and poorly differentiated cases(100%) showed strong cytoplasmic staining (High immunoreactivity score) of vimentin. So, Vimentin expression in Oral squamous cell carcinoma showed significant association with degree of differentiation of tumour. (p value:0.010)

NA-Hye Myong et al observed that normal squamous mucosa showed no immunoreactivity for vimentin.[125] Araujo et al reported that Vimentin positivity was found in many cells of high grade tumours, with poor outcome on treatment.[126] Jing ping et al observed that 6 out of 9 cases of poorly differentiated were vimentin positive with p value of 0.022.[103] Vimentin was most marked in high grade oral SCC, in tumours with basaloid phenotype was the report of the study done by Van der et al.[127]

VIMENTIN EXPRESSION IN ORAL SQUAMOUS CELL CARCINOMA WITH NODAL METASTASIS:

In present study, Vimentin expression was highly statistically significant with p value of 0.000 in oral squamous cell carcinoma presenting along with nodal metastasis.

Shuli et al found that Vimentin upregulation was strongly associated with Poor prognosis and lymph node metastasis.[128] Results of the Study done by Pei-Feng et al showed that Vimentin expression was associated with poor differentiation and lymph node metastasis.[129]

Vimentin expression was associated with higher prevalence of lymph node metastasis in head and neck squamous cell carcinoma in a study published by Mandal et al.[8]

Thus, our study proved that, as there is loss of differentiation or rise in grade of tumour, E-Cadherin expression is reduced, and Vimentin expression is increased. And there is increased expression of Vimentin when there is metastasis to the nodes.

SUMMARY AND CONCLUSION

The Concept of Epithelial mesenchymal transition is a valuable model for morphological and molecular changes observed in tumour invasion and metastasis. The changes in cellular phenotype due to EMT as shown by changes in marker protein expression and tumour aggressiveness has been proven.

This Study was carried in the Department of Pathology in Coimbatore Medical College. Total of 40 cases of Oral Squamous Cell Carcinoma cases were chosen for study and IHC markers E-cadherin and Vimentin expressions were studied.

- The Mean age of incidence of Oral Squamous cell carcinoma was 57 years.
- The Male to Female ratio of the population included in our study was 2:1.
- The most frequent sites of tumour within oral cavity was tongue(27.5%), followed by buccal mucosa and tonsillar fossa.

- The grade of tumour was moderately differentiated(57.5%) in most of the oral squamous cell carcinoma patients.

- There was significant relationship between the expression of E- Cadherin and Vimentin in Oral squamous cell carcinoma and histopathological grade.

- Significant association was noted between expression of Vimentin and presence of lymph node metastasis.

- Also, in our study there was no statistical significance between expression of E-Cadherin and presence of lymph node metastasis.

- Thus, the use of immunohistochemical stains E-cadherin and Vimentin can be used as biomarkers for predicting tumour behaviour, prognosis, survival and management of patient.

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ANNEXURE I: PROFORMA

- **Demographic details:**

  Name:

  Age (years):

  Sex:

- **Identification details:**

  Ward:

  IP/OP No:

- **Chief complaints:**

- **Clinical examination:**

  Tumour characteristics:

  Site:

  Nodal status:

- **Histopathological examination:**

  Specimen type – Wedge biopsy / Wide excision biopsy

  Carcinoma type & grade:

- **Immunohistochemistry score for E-cadherin and Vimentin:**

  Proportion score:

  Intensity score:

  Total score:

  Immunoreactivity:

ANNEXURE II: MASTER CHART

S.NO	AGE	SEX	SITE	HPE NO	NODAL STATUS		GRADE	E-CADHERIN				VIMENTIN			
					Present/ Absent	FNAC no if present		PS	IS	TS	IR	PS	IS	TS	IR
1.	60	F	Tongue	1688/17	+	1282/17	PD	2	2	4	Low	3	3	9	High
2.	63	M	Tongue	1778/17	+	1132/17	MD	3	3	9	High	3	3	9	High
3.	40	F	Buccal mucosa	2107/17	+	1256/17	MD	3	3	9	High	3	3	9	High
4.	64	M	Buccal mucosa	2848/17	+	1778/17	PD	2	2	4	Low	3	3	9	High
5.	60	F	Tongue	3346/17	+	100/18	MD	2	3	6	High	3	3	9	High
6.	40	F	Lip	3364/17	+	206/17	MD	4	3	12	High	3	3	9	High
7.	37	F	Tongue	3385/17	+	1994/17	PD	2	2	4	Low	4	3	12	High
8.	52	M	Soft palate	3397/17	-	-	MD	4	3	12	High	1	2	2	Low
9.	56	M	Tongue	3423/17	+	379/18	MD	3	3	9	High	3	3	9	High
10.	53	M	Tongue	3591/17	-	-	WD	4	3	12	High	1	2	2	Low
11.	52	M	Tonsillar fossa	3693/17	-	-	MD	4	3	12	High	1	2	2	Low
12.	60	M	Soft palate	3694/17	-	-	MD	4	3	12	High	1	2	2	Low
13.	45	F	Buccal mucosa	3695/17	-	-	WD	4	3	12	High	1	2	2	Low

14.	56	M	Buccal mucosa	3722/17	-	-	MD	3	3	9	High	2	2	4	Low
15.	68	M	Tongue	3734/17	-	-	WD	3	3	9	High	0	0	0	Negative
16.	55	F	Buccal mucosa	3736/17	-	-	MD	4	3	12	High	3	2	6	High
17.	50	M	Soft palate	4101/17	-	-	MD	4	1	4	Low	2	3	6	High
18.	65	F	Tongue	4139/17	-	-	WD	4	3	12	High	2	2	4	Low
19.	65	F	Lip	4141/17	+	604/17	PD	3	2	6	High	3	3	9	High
20.	70	M	Tonsillar fossa	4265/17	-	-	WD	4	2	8	High	0	0	0	Negative
21.	70	F	Retromolar trigone	1962/18	+	1184/18	WD	4	3	12	High	2	3	6	High
22.	58	M	Buccal mucosa	1966/18	-	-	MD	3	3	9	High	1	2	2	Low
23.	50	F	Alveolar margin	1973/18	-	-	WD	4	3	12	High	2	2	4	Low
24.	49	M	Retromolar trigone	1991/18	-	-	WD	4	3	12	High	0	0	0	Negative
25.	44	M	Tongue	1993/18	+	-	MD	4	3	12	High	2	2	4	Low
26.	56	M	Tonsillar fossa	2035/18	-	-	MD	3	3	9	High	2	3	6	High
27.	62	M	Tonsillar fossa	2042/18	+	801/18	MD	4	3	12	High	1	2	2	Low
28	60	M	Retromolar	2073/18	-	-	MD	3	3	9	High	1	2	2	Low
29.	43	F	Alveolar margin	2076/18	-	-	WD	4	3	12	High	1	2	2	Low
30.	65	M	Tonsillar fossa	2080/18	-	-	MD	3	3	9	High	2	2	4	Low

31.	45	M	Tonsillar fossa	2081/18	-	-	MD	3	3	9	High	1	2	2	Low
32.	55	M	Buccal mucosa	2083/18	-	-	WD	4	3	12	High	1	2	2	Low
33.	60	F	Floor of mouth	2131/18	+	1656/18	MD	3	3	9	High	3	3	9	High
34.	61	F	Lip	2171/18	+	1950/18	PD	2	2	4	Low	3	3	9	High
35.	40	M	Tongue	2348/18	+	1459/18	MD	4	3	12	High	3	2	6	High
36.	60	F	Lip	2838/18	+	1553/18	WD	4	3	12	High	3	2	6	High
37.	60	M	Retromolar trigone	2888/18	+	-	MD	3	3	9	High	2	3	6	High
38.	48	M	Tongue	2980/18	+	-	MD	2	3	6	High	2	3	6	High
39.	63	F	Tonsillar fossa	2982/18	+	1796/18	MD	3	3	9	High	2	3	6	High
40.	60	F	Lip	3038/18	+	1686/18	WD	4	3	12	High	3	3	9	High

HPE NO – Histopathology number; FNAC NO – Fine needle aspiration cytology number;

WD – Well Differentiated grade; MD – Moderately Differentiated grade;

PD – Poorly Differentiated grade;

PS – Proportion score; IS – Intensity score; TS – Total score; IR-Immunoreactivity.

ANNEXURE III: LIST OF ABBREVIATIONS

HPV	:	Human papilloma virus
HSV	:	Herpes simplex virus
SCC	:	Squamous cell carcinoma
LOH	:	Loss of heterozygosity
DCC	:	Deleted in colon carcinoma
CDKN2	:	Cyclin dependent kinase inhibitor 2
MTS1	:	Multiple tumour suppressor gene 1
EGFR	:	Epidermal growth factor receptor
TGF	:	Transforming growth factor
EMT	:	Epithelial mesenchymal transition
SMA	:	Smooth muscle actin
PDGF	:	Platelet derived growth factor
PAS	:	Periodic acid Schiff